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Toxicity, additive effects, and antagonism of salt solutions as indicated by growth of wheat roots

WALTER S. EISENMENGER

(WITH FOUR TEXT FIGURES)

Although the general problem of the salt relations of plants is of fundamental importance in plant physiology and has attracted the attention of investigators for more than a century, only recently have certain important phases of the problem been subjected to detailed study, and much more information of a quantitative nature will be required before it will be possible to formulate general principles. The present study has to do with the toxicity of single-salt solutions and the antagonistic effects in mixed solutions, with special reference to the influence of the total concentration of the solutions.

In presenting a brief summary of the literature on toxicity and antagonism, it will be advantageous to discuss first the investigations that have dealt with the preparation of complete nutrient solutions that support the growth and development of higher green plants. These studies were the first to be made and have yielded culture solutions that enable plants to grow to maturity under controlled conditions. They have indicated the importance of a proper set of salt proportions as well as a suitable total concentration. In the second place, studies dealing directly with the toxicity of simple solutions and antagonism in mixed solutions will be discussed.

Nutritive values of salt solutions

In the earliest work with solution cultures of higher plants, attention was directed to the development of suitable nutrient solutions for many kinds of plants. Studies were made to ascertain the kinds, forms, proportions, and total concentrations of the chemical elements that must be supplied to the roots in order to allow plants to develop to maturity in solution cultures.

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The classical researches of Sachs (1859, 1860a, 1860b), Knop (1859, 1862), and Birner and Lucanus (1866) showed that higher plants require nitrogen, sulphur, phosphorus, calcium, magnesium, potassium, and iron in an aqueous solution about their roots.

There was some discussion at this time as to whether certain other elements may not be essential. Nobbe and Siegert (1862) and Beyer (1869) reported that chlorine was necessary, and Salm-Horstmar (1856) thought that silicon and manganese were required. Following this early work, many investigators reported that several elements, especially manganese, boron, and zinc, produce stimulating effects when presented to the plant in very low concentration (Brenchley, 1910, 1914). Very recently careful studies have been made with specially purified chemicals to determine whether minute traces of these and several other elements may be essential for normal growth. As a result of these investigations strong evidence has been secured which indicates that normal growth and development of ordinary green plants does not occur in the absence of manganese (McHargue, 1923), in the absence of boron (Brenchley and Warington, 1927; Johnston and Dore, 1928; Somner, 1927; Somner and Lipman, 1926), or in the absence of zinc (Somner and Lipman, 1926). Thus manganese, boron, and zinc are no longer to be regarded merely as stimulants; they are essential mineral nutrients. It is highly probable that small quantities of a number of other elements will be found to be indispensable.

The early work of Sachs, Knop, and others indicated that plants grow better with certain sets of salt proportions than with other sets. However, the importance of a systematic testing of logically complete series of salt proportions was first emphasized by Tottingham (1914). Tottingham's investigation has been followed by important studies by several other workers who have made use of essentially the same method. Although a number of excellent nutrient solutions have been developed, much more work remains to be done before any very definite statements may be made concerning the exact salt proportions which are really *best* for a particular kind of plant under a given set of climatic conditions.

Approximately optimum total concentrations were deter-

mined in the original studies of the solution-culture method. Subsequent work by Tottingham (1914), Shive (1915), and others has indicated that best growth is generally secured with solutions having a total osmotic value of from 0.25 to 2.50 atmospheres. The exact optimum appears to depend upon the kind of plant, the salt proportions, the rate of solution renewal, and other experimental conditions. Very concentrated solutions may exert a physical influence in retarding the absorption of water by the plants, as well as a toxic effect if their components are not properly balanced; extremely dilute solutions, though practically non-toxic, may fail to supply the essential chemicals rapidly enough for best growth.

The suggestion has frequently been made that optimum solution conditions for a given kind of plant may vary according to the climatic conditions prevailing during the tests. Trelease and Livingston (1924) have actually shown that if a number of markedly different nutrient solutions are repeatedly compared by biological tests made under different climatic complexes, the relative physiological values assigned to the several solutions may be either the same or more or less different for the several tests, according to the nature and magnitude of the climatic differences dealt with.

Many improvements in solution-culture technique were introduced by Tottingham (1914) and by Shive (1915), and greater attention has recently been given to the advantages of using continuously renewed culture solutions (Trelease and Free, 1917; Trelease and Livingston, 1922; Allison and Shive, 1923; Shive and Stahl, 1927; Johnston, 1927), of selecting seeds for uniformity of weight (Trelease and Trelease, 1924), of employing statistical methods in interpreting the significance to be attached to observed differences in growth (Davis, 1921; Trelease and Trelease, 1924; Stiles, 1916), and of using chemicals that are essentially free from impurities (McHargue, 1923; Somner and Lipman, 1926; Somner, 1927; Brenchley and Warington, 1927).

Salt antagonism and physiologically balanced solutions

Wolf (1864), Boehm (1875), and Von Raumer (1883) noted that simple solutions of salts of the alkali metals or of magnesium were highly toxic to plants, and that the toxicity of such

single-salt solutions could be diminished by adding small quantities of calcium salts to the solutions. It remained, however, for Loew (1892a, 1892b) to make the first detailed study of the antitoxic action of the salts of two metallic elements—magnesium and calcium. Loew demonstrated that *Spirogyra* and the higher green plants are quickly injured by a solution which contains a magnesium salt but lacks a calcium salt. The presence of calcium in sufficient concentration prevented the toxic action of magnesium. Loew elaborated a hypothesis to explain the toxic effect and its counteraction, and in later papers he emphasized the great importance of a proper balance between magnesium and calcium in soil as well as in solution cultures; but he appears to have regarded these two elements as in a category by themselves, for he did not extend his hypothesis to include salts of other elements (Loew and May, 1901).

It is to Loeb (1900, 1902, 1906, 1912) that we are indebted for the development of the modern conception of physiologically balanced solutions. He was the first investigator to report extensive studies of salt antagonism and to distinguish clearly between balanced solutions and nutrient solutions. Loeb discovered that young fish (*Fundulus*) soon die if placed in a solution of NaCl of the concentration in which this salt is contained in sea water. When KCl and $CaCl_2$ were added to the solution in the right proportions, the fish could live indefinitely. Since the fish could also live for a long period of time in distilled water, Loeb concluded that KCl and $CaCl_2$ serve to render the NaCl harmless but do not act as nutritive substances.

Antagonistic salt action was demonstrated by Loeb in an equally striking manner by experiments with the eggs of *Fundulus*. The fertilized eggs of this marine fish were observed to develop normally in sea water or in distilled water, but if placed in an m/2 NaCl solution, they died without forming an embryo. If, however, a trace of a calcium salt was added to the m/2 NaCl solution, the toxicity was greatly diminished and the eggs lived long enough to form embryos. The valence rule was established by further tests in which it was found that the toxic effects of salts of monovalent cations (Na, Li, K, NH_4) could be prevented by the addition of a small amount of a salt of almost any bivalent cation (Ca, Ba, Sr, Zn, Pb, Co, Ni) or

by any one of a number of trivalent cations. Anions, however, were observed to be practically without antagonistic action.

Antagonism was considered by Loeb to depend upon an action of both salts on the egg membrane, whereby the membrane becomes nearly impermeable to both salts. The toxicity of each salt in simple solution was assumed to be due to its rapid diffusion through the membrane.

Kearney and Cameron (1902) reported an extensive series of tests on lethal concentrations of simple and mixed solutions for *Lupinus albus*. They found that in the mixed solutions the toxic effect of the more injurious component was greatly diminished, or the lethal concentration of the more toxic salt was markedly increased. The addition of sodium ions to a solution containing magnesium ions in most instances markedly weakened the toxic action of the latter; and the addition of calcium ions to solutions containing either sodium or magnesium ions nearly always counteracted to an extraordinary degree the injurious effects of the sodium or magnesium ions.

Osterhout, in a series of investigations with plants, has confirmed and greatly extended the work of Loeb on animals. He (1906) found that each of the salts of the sea water was toxic to marine algae when it alone was present in solution; but the toxic effects were mutually counteracted in a mixture of these salts in the proper proportions. The results of further experiments by Osterhout (1907) with fresh water algae, liverworts, *Equisetum*, and several species of flowering plants were in close agreement with those obtained from his study of marine plants, and showed that physiologically balanced solutions have the same fundamental importance for plants as for animals.

Osterhout (1907) emphasizes the view that the injurious effects of single-salt solutions cannot be due merely to starvation, for distilled water is less harmful than simple solutions. Furthermore, he emphasizes the distinction between balanced solutions and nutrient solutions. A nutrient solution must contain all substances needed for nutrition; it may be used in such dilute form that none of its components would exert a toxic action even if the other constituents were removed. A balanced solution, on the other hand, is composed of salts that would be toxic in simple solution; the components of the

balanced solution do not necessarily have a nutritive value. If the concentration of a complete nutritive solution is increased, a point is reached where some or all of the components begin to exert their individual toxic effects, whereupon it may become necessary to inhibit these effects by proper adjustment of the relative proportions of the substances present or by the addition of other substances.

In a later series of papers Osterhout (1914a, 1914b, 1915, 1918) calls attention to the fact that in order to express antagonism quantitatively it is necessary to know the *additive effect*—that is, the total toxic effect that the solution would have if no antagonism existed and if each salt exerted its toxic effect independently. Osterhout illustrates the use of the additive effect by means of some data representing the growth of wheat roots after thirty days in simple and in mixed solutions of NaCl and $CaCl_2$. By comparing the additive effect with the effect of the mixed solution, it may easily be observed whether the toxicity of the two salts in the mixed solution is unaltered (the same as the additive effect), whether the toxicity is diminished (less than the additive effect), or whether the toxicity is increased (greater than the additive effect). Moreover, the additive effect, if known, serves as the basis for an accurate quantitative measurement of antagonism. In cases where equally toxic simple solutions of the two salts remain equally toxic when diluted to the same degree, Osterhout has found it convenient to employ mixtures of equally toxic solutions. In such cases, the additive effect of every mixture is the same as the effect of either simple solution. If, for example, growth is more rapid than in either of the simple solutions, this increase in growth represents antagonism.

Osterhout (1915, 1918) has pointed out that if equally toxic simple solutions do not remain equally toxic when diluted to the same degree, the additive effect cannot be easily determined. A value of the additive effect may be assigned, however, if it is possible to express approximately the effect of one salt in terms of the other salt.

That difficulty may be experienced in attempting to estimate the additive effect has been emphasized by Trelease and Trelease (1926a). They point out the fact that a simple graphical test may be made to determine whether equally toxic

simple solutions will remain equally toxic when diluted to the same degree. This relation will exist only if growth curves (or other curves representing plant response) have the same slope when plotted with a logarithmic scale for concentrations. It did not exist for their data for root elongation in simple solutions of KNO_3, $Ca(NO_3)_2$, and $Mg(NO_3)_2$, since the growth curves had different slopes. Furthermore, Barton and Trelease (1927) showed that extremely pronounced differences were apparent between the slopes of curves representing growth in simple solutions of $MnCl_2$ and $Ca(NO_3)_2$. In such extreme cases, it seems impossible to attempt to estimate additive effects in mixed solutions.

Alterations in the form of antagonism curves as affected by dilution are discussed by Osterhout (1914c). Although he does not give the results of specific experiments, he gives diagrams representing generalized types of curves. These show that with very concentrated solutions the antagonism curve exhibits a distinct maximum corresponding to a clearly defined optimum set of salt proportions. But as the solution becomes more dilute, the antagonism curve becomes flatter, and at a still greater dilution it tends to become a horizontal straight line. In general, he says, growth in strong solutions furnishes a much more satisfactory criterion of antagonism than growth in weak solutions.

McCool (1913) studied the effects of a large series of salts on the growth of young wheat and pea seedlings. The following cations (arranged in the order of decreasing toxicity) were studied: Ba, Sr, NH_4, Mg, Na, and K. Mutual antagonism was observed between Mg and NH_4, K and Ba, Mg and Ba; but Ca was most effective in preventing toxic action. McCool also reports that the toxicity of Mn ions was counteracted by Ca, K, Na, and Mg ions, and that mutual antagonism exists between the Mn ion and each of the following: K, Na, and Mg.

Valuable discussions of the literature are given by Stiles and Jörgensen (1914) and by Stiles (1923).

Hansteen Cranner (1910, 1914) reported observations of the specific effects of a number of cations on root development of wheat. His results, in general, are in agreement with those of Osterhout. Antagonism, even in solutions as dilute as 0.0013M, was recorded between K and Ca and between K and Mg.

Szücs (1912) showed that $AlCl_3$ decreased the toxicity of $CuSO_4$. He used an ingenious criterion of toxicity—namely, the inability of injured roots to exhibit geotropic response after immersion in toxic solutions. Also, using duration of life of *Spirogyra*, he obtained evidence that KNO_3 had a slight antagonistic action toward quinine hydrochloride, $Ca(NO_3)_2$ had a more pronounced effect, and $Al(NO_3)_3$ the greatest effect. This agreed with Loeb's conclusion that the antagonistic power is a function of the cation valence. Moreover, microchemical observation indicated that the diminished toxicity was due, as Loeb had suggested, to the hindrance of the entrance of the poison.

Hawkins (1913) made a careful study of the influence of calcium, magnesium, and potassium nitrates upon the toxicity of certain heavy metals toward the spores of *Glomerella cingulata*. Marked antagonism, apparently resulting from a simultaneous action of the two salts upon the spores, was observed with most mixed solutions. The results with $Cu(NO_3)_2$ are especially interesting since it was found that in order to counteract the toxicity of $Cu(NO_3)_2$ the molecular ratio of $Ca(NO_3)_2$ to $Cu(NO_3)_2$ needed to be increased from 0.63 to 6.25 when the concentration of $Cu(NO_3)_2$ was increased from 0.0001M to 0.0080M. Thus, favorable proportions of the two salts changed with alterations of concentration—an observation which is apparently not consistent with the results obtained by several investigators, including Osterhout.

As a result of an extensive series of tests in which electrical conductivity of tissues was used as the criterion of antagonism, Osterhout (1922) has developed an elaborate hypothesis to explain antagonism. He assumes that the antagonistic salts combine with some constituent of the protoplasm to form a compound, at a surface (the external surface of the cell or internal surfaces). On account of antagonistic action, the salts in a balanced solution penetrate living cells more slowly than do salts in an unbalanced solution, and the slow penetration has no unfavorable influence on the life processes so long as the substances within the cell remain properly balanced. Loeb (1906) and Szücs (1912) also explained antagonism as due to the fact that antagonistic substances tend to prevent each other from entering the cell.

Osterhout's hypothesis is in harmony with his observation that while variations in total concentration affect the form of the antagonism curve, they do not in general affect the proportions which are most favorable for life processes.

Osterhout (1922) has also formulated a hypothesis which enables one to predict what substances (including both electrolytes and non-electrolytes) will antagonize each other. This hypothesis states that a substance which causes a decrease of electrical resistance (or of permeability) of plant tissues will antagonize any substance which produces an increase of electrical resistance (or permeability) followed by a decrease. The degree of antagonistic action could also be predicted by observing the amount of decrease of resistance produced by the salts.

Object of this investigation

The present study was planned in order to obtain further information concerning the toxicity of simple solutions and antagonism in solutions containing two salts. Special attention was given to the influence of total concentration in mixed solutions. It was thought that such a study, besides adding directly to our knowledge of toxic and balanced solutions, would help explain the action of complete nutrient solutions for plants, since physiological balance is one of the primary requirements of a suitable culture solution.

As a criterion of toxicity, the growth of the roots of germinating wheat was employed. The initial behavior of germinating seeds involves less complexity, within as well as outside the organism, than is involved in later phases of growth. The complicated question of illumination may be avoided, since such tests can be carried out in darkness. Furthermore, the experiment period can be relatively short, thus practically avoiding many of the alterations in organism that increase with time. During the very early seedling stage, the plants are well supplied with organic food, and the initial supply of salts in the seedlings is sufficient to prevent the appearance of starvation symptoms in roots which are grown in distilled water. Nevertheless this small supply of salts does not seem to be great enough to influence significantly the toxic action of a solution in which the roots are immersed. The protoplasm of the roots appears to be readily accessible to the toxic agent,

and retardation of root growth furnishes an excellent criterion of toxicity.

The three salts, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ were chosen for this study because of their importance in the preparation of nutrient solutions for higher plants. When all three of these salts are employed in a culture solution, they supply, in suitable forms, the mineral elements needed in largest quantities by higher green plants. Small amounts of iron, manganese, boron, zinc, and perhaps other elements are also required, but these generally may be supplied in concentrations so low as to have no important influence on the physiological balance of the solution.

In an extensive study of the growth of wheat plants in culture solutions containing ordinary 'chemically pure' grades of these three salts, Shive (1915) found that growth during the first four weeks after germination was as satisfactory with this relatively simple solution as with solutions containing a greater number of salts. Other workers have also reported that solutions containing these three salts give excellent growth of plants during the seedling phase of development (Trelease and Livingston, 1924; Trelease and Trelease, 1924).

Although six types of three-salt solutions may be made to contain the six main ions (Livingston, 1919; Morita and Livingston, 1920), the salts used in the present study appear to possess some advantages over those used in the other types of solutions. It is therefore of considerable importance to obtain as much information as possible concerning the growth responses to simple and mixed solutions of these three salts. Such information should be of fundamental importance in many studies of the growth of higher plants in artificial culture media.

In the investigation here reported a study was made of the toxicity of simple solutions of KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ in which the concentration of each salt ranged from 0.00004M to 0.24M. Antagonism was tested by means of mixed solutions containing pairs of these salts—namely, $KH_2PO_4+Ca(NO_3)_2$, $Ca(NO_3)_2+MgSO_4$, and $KH_2PO_4+MgSO_4$. For each pair of salts five total concentrations were employed (0.002M, 0.006M, 0.02M, 0.06M, and 0.12M). And with each total concentration eleven sets of molecular pro-

portions of the two salts were used, as follows: 0+100, 2+98, 5+95, 15+85, 30+70, 50+50, 70+30, 85+15, 95+5, 98+2, and 100+0.

METHODS

The methods employed were essentially the same as those described in several papers from this laboratory (Trelease and Trelease, 1925, 1926a, 1926b; Barton and Trelease, 1927). A pure line of spring wheat was used (Marquis, Saskatchewan no. 70, secured through the courtesy of Professor Manley Champlin of the University of Saskatchewan). The seeds were sorted to secure as great as possible uniformity of size and type. They were germinated on moist filter paper in glass culture dishes in a dark room.

For each culture two Pyrex beakers (usual or tall form, without spout) were used. The smaller was of 300 cc. capacity, and the larger of 600 cc. capacity. Over the top of the small beaker was stretched a piece of paraffined bobbinet, which was secured below the rim by a ligature of paraffined linen thread. The smaller beaker was placed inside the larger one, and the culture solution was poured in until the liquid levels inside and outside the smaller beaker were even at its top.

When the primary roots of the seedlings had an average length of about 6 mm., selected seedlings were placed on the bobbinet so that the roots dipped into the culture solution. Duplicate cultures, each of twenty-five plants, were used for each experimental solution. The cultures were placed on a rotating table (Shive, 1915) in a dark room, and the seedlings were allowed to grow until the primary roots of the control culture had acquired a length of about 90 mm., or until these roots had elongated about 84 mm. (90 mm. minus 6 mm.).

The length of the primary root of each individual plant was then recorded, and the average length of the roots of each culture was computed. From this length was deducted the average length of the roots when the seedlings were taken from the germinating dish. This difference constituted the average elongation for the culture. The growth data presented in this paper are relative values. Each relative growth value was obtained by dividing the average elongation of a given culture

by the average elongation of the control culture and multiplying this quotient by one hundred.

The average minimum temperature during the growth of the seedlings was 16°C., and the average maximum temperature

TABLE 1

Growth in simple solutions of potassium dihydrogen phosphate[a]

MOLECULAR CONCENTRATION	INDIVIDUAL TESTS, 25 SEEDLINGS EACH	AVERAGE
0.00004	68, 61	65
0.00010	62, 55	59
0.00012	50, 48	49
0.00030	48, 46, 43, 37	44
0.00040	43, 38	41
0.00060	47, 47	47
0.00090	40, 35	38
0.00100	46, 37, 41, 39	41
0.00120	36, 36	36
0.00170	41, 39	40
0.00180	38, 37	38
0.00240	40, 39	40
0.00300	43, 42, 38, 36, 33, 31	37
0.00400	41, 37	39
0.00510	42, 38	40
0.00600	38, 34, 40, 34	37
0.00900	32, 29	31
0.01000	32, 31	32
0.01200	44, 40	42
0.01700	36, 33	35
0.01800	35, 33, 34, 34	34
0.03000	23, 19	21
0.03600	19, 16	18
0.04000	20, 17	19
0.05100	8, 6	7
0.06000	4, 4	4
0.10200	3, 2	3
0.12000	2, 2	2
0.24000	2, 0	1

[a] The average value for growth in distilled water was 84. This is based on the following data for cultures of 25 seedlings each: 79, 71, 86, 79, 92, 92, 73, 66, 78, 74, 93, 87, 74, 63, 92, 79, 100, 95, 72, 67, 86, 79, 98, 89, 102, 97, 101, 97, 80, 77.

was 21°C. The time required for the roots of the control solutions to acquire a length of 84 mm. averaged 97 hours. The higher the temperature, the shorter was the culture period.

Four control cultures of 25 plants each were used. Also, two distilled water cultures of 25 plants each were included in each series. The composition of the control solution was as follows: 0.02 M KH_2PO_4, 0.02 M $Ca(NO_3)_2$, and 0.02 M $MgSO_4$.

The water used was obtained from a Barnstead still. The magnesium sulphate and calcium nitrate were Powers-Weightman-Rosengarten 'analytical' chemicals, and the potassium dihydrogen phosphate was of Merck's 'blue-label' grade. The experimental work was performed in the laboratory adjacent to the greenhouse at Columbia University, during the period from November, 1925, to March, 1926, inclusive.

RESULTS OF TESTS WITH SINGLE-SALT SOLUTIONS

The relations between growth rates and volume-molecular concentrations of single-salt solutions are shown in the graphs of figure 1, plotted from the data of table 1. For each of the three salts, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$, the results of five series of cultures are included in the figure. The abscissas represent volume-molecular concentrations plotted on a logarithmic scale, and the ordinates represent growth rates expressed as percentages of the growth rate in the three-salt standard or control solution. In each case all of the observed points are shown and a smoothed curve has been drawn by inspection to represent what appears to be the general trend of the points. The smoothed curve is not defined as precisely as might be desired, especially for concentrations below 0.001 M; but it may be expected to represent approximately the true relationships. It will be observed that the three graphs differ greatly in form; each will be considered in detail in the following sections of this paper.

Solutions of potassium dihydrogen phosphate. The graph for KH_2PO_4 in figure 1 falls gradually for concentrations from 0 M to 0.0003 M. From a concentration of 0.0003 M to one of 0.02 M the downward slope is so slight as to be barely perceptible. This is followed, however, by a pronounced decline between 0.02 M and 0.06 M, and by a gradual fall from 0.1 M to 0.24 M. Even the lowest concentration, 0.00004 M, had a pronounced growth-retarding effect, since the growth rate which it permitted was 36 per cent lower than that for the control solution and 22 per cent lower than that for distilled water.

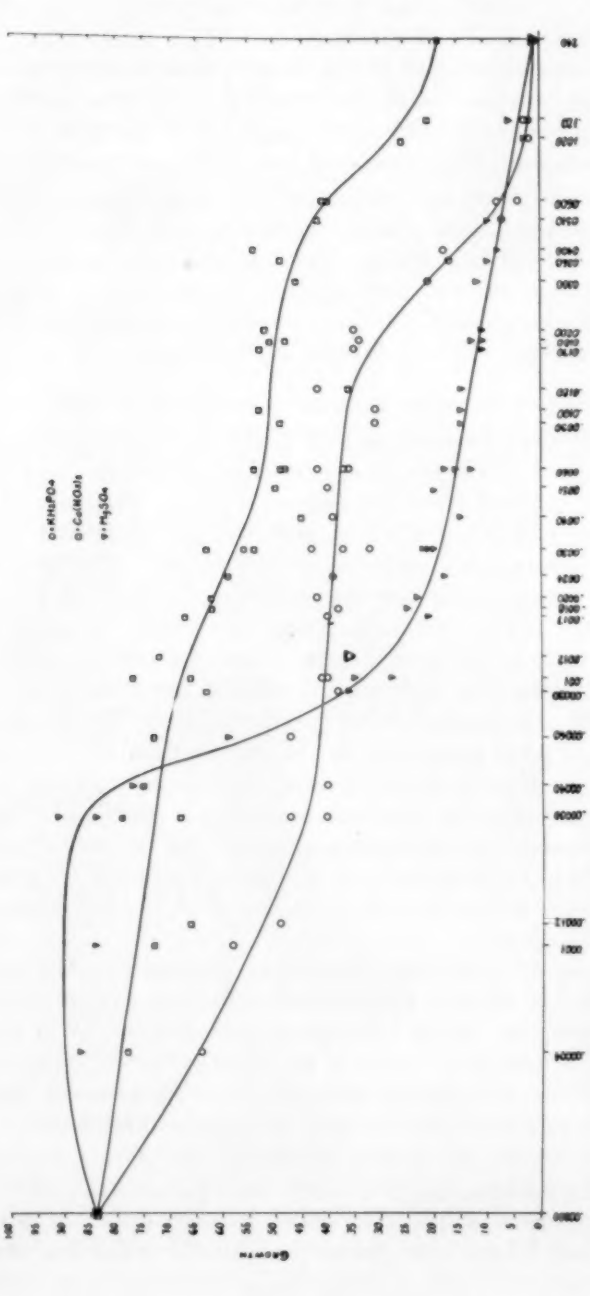

Fig. 1. Elongation of wheat roots in simple solutions of KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$. Ordinates represent percentages of elongation for standard solution; abscissas represent volume-molecular concentrations plotted on a logarithmic scale.

The marked toxic effect of this salt in very dilute solutions may perhaps be attributed to the relatively high hydrogen-ion concentration of these solutions.

Solutions of calcium nitrate. Minor fluctuations in the uniform downward slope of the curve for $Ca(NO_3)_2$ may be noted in the somewhat steeper slope for concentrations within the range of 0.001 M to 0.006 M and in the rather abrupt fall in the curve for concentrations between 0.06 M and 0.12 M. Growth in the most dilute solution (0.00004 M) was about 79 per cent as rapid as in the control solution and nearly as rapid as in distilled water, while growth in the most concentrated solution (0.24 M) was about 18 per cent as rapid as in the control solution.

Solutions of magnesium sulphate. The graph for $MgSO_4$ rises within the range from 0 M to 0.0003 M. It then falls abruptly from 0.0003 M to 0.001 M, and finally exhibits a gradual decline within the range from 0.001 M to 0.24 M.

The initial rise in the curve, for very dilute solutions of $MgSO_4$, is of special interest, since it appears to be related to a principle frequently stated in physiological literature—namely, that dilute solutions of a substance which is toxic in higher concentrations may be expected to have stimulating effects (see, for example, Richards, 1897, 1910). Very dilute solutions of $MgSO_4$ appear to induce slightly more rapid growth than occurs in distilled water. Stimulation of growth by very dilute solutions of other salts has been reported by Jensen (1907), Brenchley (1914), Winslow and Falk (1923), Hotchkiss (1923), Coupin (1898), Kearney and Cameron (1902), Barton and Trelease (1927), and other workers. With the exception of the initial rise, the general outline of the growth curve for $MgSO_4$ is in accord with that for $Mg(NO_3)_2$ given by Trelease and Trelease (1926a). It should be noted, however, that $MgSO_4$ appears to be somewhat less toxic than $Mg(NO_3)_2$—a difference which may be related to a lower degree of ionization of $MgSO_4$.

General forms of growth curves. Attention may be called to certain general features of figure 1. While the curves for $Ca(NO_3)_2$ and KH_2PO_4 exhibit a marked similarity in form, that for $MgSO_4$ differs greatly from either of these.

It will be observed that throughout the range of concentrations KH_2PO_4 is considerably more toxic than $Ca(NO_3)_2$, this difference being especially pronounced in the very low and in the highest concentrations. Below a concentration of about

TABLE 2

Growth in simple solutions of calcium nitrate

MOLECULAR CONCENTRATION	INDIVIDUAL TESTS, 25 SEEDLINGS EACH	AVERAGE
0.00004	82, 74	78
0.00010	73, 72	73
0.00012	71, 61	66
0.00030	70, 65, 79, 79	73
0.00040	78, 73	76
0.00060	75, 70	73
0.00090	67, 60	64
0.00100	79, 75, 69, 64	72
0.00120	76, 67	72
0.00170	71, 63	67
0.00180	68, 55	62
0.00240	59, 58	59
0.00300	56, 52, 61, 50, 63, 62	57
0.00400	46, 44	45
0.00510	54, 46	50
0.00600	54, 54, 49, 48	51
0.00900	49, 48	49
0.01000	56, 50	53
0.01200	42, 31	37
0.01700	59, 48	54
0.01800	52, 44, 52, 50	50
0.03000	48, 45	47
0.03600	50, 47	49
0.04000	54, 54	54
0.05100	42, 42	42
0.06000	42, 38	40
0.10200	26, 26	26
0.12000	22, 21	22
0.24000	20, 18	19

0.0004 M, $MgSO_4$ is less toxic than either of the other salts. For a limited range above this concentration (between points where the curve for $MgSO_4$ intersects those for $Ca(NO_3)_2$ and KH_2PO_4), $MgSO_4$ is less toxic than KH_2PO_4 but more toxic than $Ca(NO_3)_2$. Then for a wide range of concentrations $MgSO_4$

is much more toxic than either of the other salts. Finally, it should be noted that above a concentration of about 0.06 M, KH_2PO_4 and $MgSO_4$ are about equally toxic, while $Ca(NO_3)_2$ remains much less toxic than the other salts.

TABLE 3

Growth in simple solutions of magnesium sulphate

MOLECULAR CONCENTRATION	INDIVIDUAL TESTS, 25 SEEDLINGS EACH	AVERAGE
0.00004	90, 84	87
0.00010	87, 81	84
0.00012	111, 95	103
0.00030	90, 78, 91, 91	88
0.00040	80, 74	77
0.00060	60, 58	59
0.00090	39, 34	37
0.00100	30, 26, 35, 34	31
0.00120	39, 34	37
0.00170	22, 20	21
0.00180	26, 24	25
0.00240	20, 17	19
0.00300	23, 21, 20, 20, 21, 21	21
0.00400	16, 15	16
0.00510	21, 18	20
0.00600	16, 16, 13, 13	15
0.00900	16, 15	16
0.01000	16, 14	15
0.01200	15, 15	15
0.01700	12, 10	11
0.01800	13, 13, 11, 11	12
0.03000	13, 11	12
0.03600	10, 10	10
0.04000	8, 8	8
0.05100	10, 10	10
0.06000	7, 6	7
0.10200	3, 3	3
0.12000	6, 5	6
0.24000	1, 1	1

The pronounced differences in form exhibited by the three curves suggest that the relation between toxicity and concentration is specific for each salt. This does not mean, of course, that a study of many salts may not show that they

fall into a limited number of general classes with respect to their growth retarding effects.

Attention should be called to the rather steep fall which occurs in each of the three graphs—for $MgSO_4$ beginning at 0.0004 M, for KH_2PO_4 at 0.02 M, and for $Ca(NO_3)_2$ at 0.06 M. With $MgSO_4$ and KH_2PO_4, the abrupt decline appears to be due to chemical rather than osmotic effects, since the concentrations at which it occurs are below those considered to have any marked osmotic influence. With $Ca(NO_3)_2$, however, it seems possible that the decline above 0.06 M may be related to the high osmotic concentrations of these solutions.

Comparison of concentrations corresponding to certain ranges of growth rates. The range of growth rates (represented by ordinates in figure 1) may be divided into four parts: (a) a range of stimulation or of rates higher than that in distilled water, (b) a range of high growth rates (from 72 to 84 per cent of the rate for the control solution), (c) a range of medium growth rates (from 41 to 72 per cent of the rate for the control solution), and (d) a range of low growth rates (from 0 to 41 per cent of the rate for the control solution).

It will be seen that $MgSO_4$ is the only salt which induces growth rates which can be characterized as in the range of stimulation. Within the range of high growth rates, the order of toxicity of the salts (from most toxic to least toxic) is KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4$. For the range of medium growth rates, the order is KH_2PO_4, $MgSO_4$, $Ca(NO_3)_2$, and for low growth rates, the order is $MgSO_4$, KH_2PO_4, $Ca(NO_3)_2$. Thus, $MgSO_4$ shifts from the position of least toxicity to that of medium toxicity and finally to that of highest toxicity, as we consider successively ranges of stimulation and high growth rates, medium growth rates, and low growth rates.

Comparison of molecular concentrations producing equal growth rates. If any pair of curves in figure 1 (in which a logarithmic scale is used for concentrations) had the same slope, this would signify the existence of a constant ratio between molecular concentrations of solutions producing equal growth rates (or having equal growth retarding effects). Since the slopes are different, it is of interest to see to what extent the ratios differ. This is brought out by the data of table 4, which have been derived from figure 1. Comparing single-salt solu-

tions of $Ca(NO_3)_2$ and KH_2PO_4, it will be observed that the number of molecules of $Ca(NO_3)_2$ equivalent to one molecule of KH_2PO_4 is at first small, that it increases to a maximum and that it finally becomes small again, as we pass from relatively non-toxic to very toxic solutions. Similar relations hold for comparisons of $Ca(NO_3)_2$ with $MgSO_4$ and of KH_2PO_4 with $MgSO_4$.

For relatively non-toxic solutions, 3 molecules of $Ca(NO_3)_2$ are equivalent in toxicity to one molecule of KH_2PO_4. For increasingly toxic solutions, the number of molecules of $Ca(NO_3)_2$ equivalent in toxicity to one molecule of KH_2PO_4 becomes

TABLE 4

Molecular concentrations of single-salt solutions required to produce various equal relative growth rates; also a comparison of these concentrations for pairs of salts (Data derived from graphs of figure 1)

RELATIVE GROWTH RATE	MOLECULAR CONCENTRATIONS OF SINGLE-SALT SOLUTIONS			NO. OF MOLECULES OF $Ca(NO_3)_2$ EQUIVALENT TO ONE MOLECULE OF KH_2PO_4	NO. OF MOLECULES OF $Ca(NO_3)_2$ EQUIVALENT TO ONE MOLECULE OF $MgSO_4$	NO. OF MOLECULES OF KH_2PO_4 EQUIVALENT TO ONE MOLECULE OF $MgSO_4$
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$			
80	.00001	.00003	.00040	3	0.1	0.03
70	.00003	.00069	.00048	23	1.4	0.06
60	.00006	.00240	.00056	40	4.3	0.11
50	.00015	.01738	.00065	116	26.7	0.23
40	.00141	.06026	.00081	43	74.5	1.74
30	.01862	.09333	.00111	5	84.1	16.77
20	.03162	.19950	.00251	6	79.5	15.84
10	.05129	.....	.02630	...	....	1.95

greater, owing to the fact that the toxicity of KH_2PO_4 increases more rapidly than does the toxicity of $Ca(NO_3)_2$. A maximum is attained for solutions allowing a growth rate of 50 per cent of that of the control culture; for these solutions 116 molecules of $Ca(NO_3)_2$ are equivalent in toxicity to one molecule of KH_2PO_4. For still more toxic solutions the number of molecules of $Ca(NO_3)_2$ equivalent to one molecule of KH_2PO_4 diminishes rapidly, so that for relatively very toxic solutions, 5 molecules of $Ca(NO_3)_2$ are equivalent to one molecule of KH_2PO_4.

Although similar relations are shown for the other pairs of salts, the maximum number of molecules of $Ca(NO_3)_2$ equivalent in toxicity to one molecule of $MgSO_4$ is indicated for a

solution allowing only 30 per cent of the growth rate for the control culture. And the maximum number of molecules of KH_2PO_4 equivalent to one molecule of $MgSO_4$ is shown for a solution corresponding to the same growth rate.

The data of table 4, considered in connection with figure 1, suggest that growth equivalent to that in distilled water may be obtained with very dilute solutions of all molecular species. As we pass from slightly to highly toxic solutions, differences in toxicity of any two molecular species are at first small, but they become more pronounced and finally less pronounced.

General relations between concentration and toxic action. Several schemes were used for plotting growth rates corresponding to the concentrations of the three salts tested in the present study. Growth rates and also logarithms of growth rates were plotted against volume-molecular concentrations and against logarithms of these concentrations. These curves failed, however, to reveal sufficient evidence to justify the formulation of any simple rule regarding the relationship of toxicity to concentration, over the wide ranges of concentration tested (the maximum concentration of each salt being 6,000 times as great as the minimum). Such a rule would need to be qualified by numerous correction factors, each of which would apply to a relatively small range of concentration. For any given salt the relations between toxicity and concentration appear to be very complex, and the relations for one salt are not the same as those for another.

An examination of the literature shows that a relatively simple relation between concentration and toxic action has been derived from some studies. Thus, Chick (1908), working on the effect of disinfectants on the period of bacterial survival, was able to state the relation in simple mathematical terms. A process of disinfection was found to obey the laws of a monomolecular chemical reaction, characterized by a definite velocity constant; and a study of the velocity constants for a wide range of concentrations of the disinfectant showed that the velocity of disinfection is a power function of the concentration. Similar relations have been reported by Krönig and Paul (1897), Paul, Birstein, and Reuss (1910), and Falk and Winslow (1926). Also, Cook (1926) found that the speed of the toxic action of salts of heavy metals (copper, silver, and mercury) on the

respiration of *Aspergillus niger* varied as a constant power of the concentration. It seems possible that, when proper data are available, similar relations may be found to exist between concentration and toxic action on root growth.

Relations between nature of salt and toxic action. Since only three salts were used in the present study, no adequate data are given for discussing relations between the nature of the salts and their toxic action. It will be of interest, nevertheless, to refer to the work of Matthews (1904), who concluded from his studies that the toxic action of metallic salts is inversely proportional to the solution tensions of the metals in question and is thus related to the ease with which the ions part with their electrical charges. He suggested an empirical formula for calculating the approximate fatal dose of one salt when the fatal dose of another salt and the solution tensions of both metals are known.

Salts of the heavy metals have generally been found to be more toxic than those of the lighter metals. From studies of the toxic action of metallic salts on *Spirogyra*, Scarth (1924) reported that in some series of chemically allied elements (such as Ba, Sr, Ca; Hg, Cd, Zn; Ce, Yt) toxicity varies directly with atomic weight. In the alkali metals, however, he found the reverse relation.

It is implied in the rules just cited that a list may be made which shows a series of salts in the order of increasing toxicity (each salt in the list being less toxic than those following it). The same idea is involved in the conclusion of Le Renard (1912), who reported that the degree of toxicity of any solution may be calculated if we know the coefficient of toxicity of the salt in question and its concentration in the solution. Similarly, Osterhout (1915) states that equally toxic solutions of two different salts will in most cases remain equally toxic if both solutions are diluted to the same degree. It should be emphasized, however, that the results secured in the present study show that the order of toxicity of several salts is not constant but that it varies according to the concentrations considered. Thus, for low concentrations the order of decreasing toxicity is KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4$; for medium concentrations it is KH_2PO_4, $MgSO_4$, $Ca(NO_3)_2$; and for high concentrations it is $MgSO_4$, KH_2PO_4, $Ca(NO_3)_2$.

Appearance of roots. Roots that had grown in solutions of $MgSO_4$ of high concentration were bent and had a dark brown color, the coloration being especially conspicuous near the upper part of the roots where they join the stem; the entire lower part of each root showed signs of flaccidity, sloughing off, and general disorganization. In solutions of $Ca(NO_3)_2$ the roots were white and normal in appearance, except in the highest concentration, where they seemed to be slightly swollen and exhibited an increased tendency to break when handled. In solutions of KH_2PO_4 of concentrations higher than 0.02 M the roots were swollen and suggestive of bits of gelatine; yet they were brittle and easily broken.

The maximum number of roots developed in $MgSO_4$ solutions was five, rarely seven. In solutions of $Ca(NO_3)_2$ and KH_2PO_4 one or three, or rarely five, roots developed.

Root hairs were especially conspicuous on roots which had grown in $Ca(NO_3)_2$ solutions at concentrations of 0.04 M and lower. The root hairs were not only very long but they were densely crowded together. Similar observations are recorded by Magowan (1908), Trelease and Trelease (1926), and Farr (1927).

RESULTS OF TESTS WITH TWO-SALT SOLUTIONS

Mixed solutions of potassium dihydrogen phosphate and calcium nitrate

General features of graphs. The graphs of figure 2, representing elongation of wheat roots in mixed solutions of KH_2PO_4 and $Ca(NO_3)_2$, are round-topped and rather regular and symmetrical in form. An exception is evident in the case of the most concentrated mixtures, 0.12 M, for which the curve is unsymmetrical, having a distinct peak for mixtures with very high proportions of the potassium salt. In a general way, the relations are similar to those illustrated by Trelease and Trelease (1926a) for mixtures of the nitrates of potassium and calcium having a total concentration of 0.06 M. No very conspicuous change in the shape of the curves of figure 2 is evident as the concentration changes from 0.002 to 0.06 M; but, as just mentioned, the curve for mixtures of the highest molecular concentration is quite different in shape from the others, having a distinct maximum for mixtures containing

high proportions of KH_2PO_4. It will be observed that the curves for the three lowest concentrations rise to about equal heights, reaching or approaching closely a value of 100 (the growth of the roots in the standard solution); the curve for 0.06 M does not rise so high, however, and that for 0.12 M is still lower in height. Thus, for any given set of salt proportions, growth tends to be inversely related to the total concentration of the solution.

TABLE 5

Growth in mixed solutions of potassium dihydrogen phosphate and calcium nitrate

PROPORTIONS		0.002 M SERIES			0.006 M SERIES			0.02 M SERIES			0.06 M SERIES			0.12 M SERIES		
KH_2PO_4	$Ca(NO_3)_2$	A	B	Av.	A	B	Av.	A	B	Av.	A	B	Av.	A	B	Av.
0	100			62			49			52			41			21
2	98	60	58	59	62	60	61	87	79	83	60	57	59	28	28	28
5	95	70	68	69	74	68	71	94	92	93	64	60	62	26	25	26
15	85	82	80	81	93	88	91	97	94	96	73	70	72	18	17	18
30	70	92	89	91	104	96	100	91	91	91	81	75	78	16	12	14
50	50	101	99	100	97	93	95	87	83	85	82	81	82	9	8	9
70	30	96	94	95	100	93	97	90	88	89	83	83	83	30	22	26
85	15	69	68	69	102	95	99	85	82	84	71	65	68	72	69	71
95	5	58	52	55	91	81	86	83	81	82	61	59	60	68	65	67
98	2	54	52	53	76	73	75	77	73	75	55	54	55	57	54	56
100	0			42			42			35			8			3

Critical ranges and ranges of indifference. In general each curve for growth in the mixed solutions exhibits a steep slope at the extreme left and the extreme right. These steep portions correspond to salt mixtures in which the relative proportion of one of the salts to the other is very low. These may be considered as critical ranges of salt proportions, in which a slight difference in the ratio of one salt to the other is correlated with a very pronounced difference in the growth rate. Within these critical ranges the roots are very sensitive to small differences in salt proportions. Between the critical ranges of salt proportions, there may be distinguished a range of indifference, within which marked differences in salt proportions may occur without any corresponding differences in rates of growth. Optimum salt proportions, where it is possible to distinguish these, of course lie in this range of indifference.

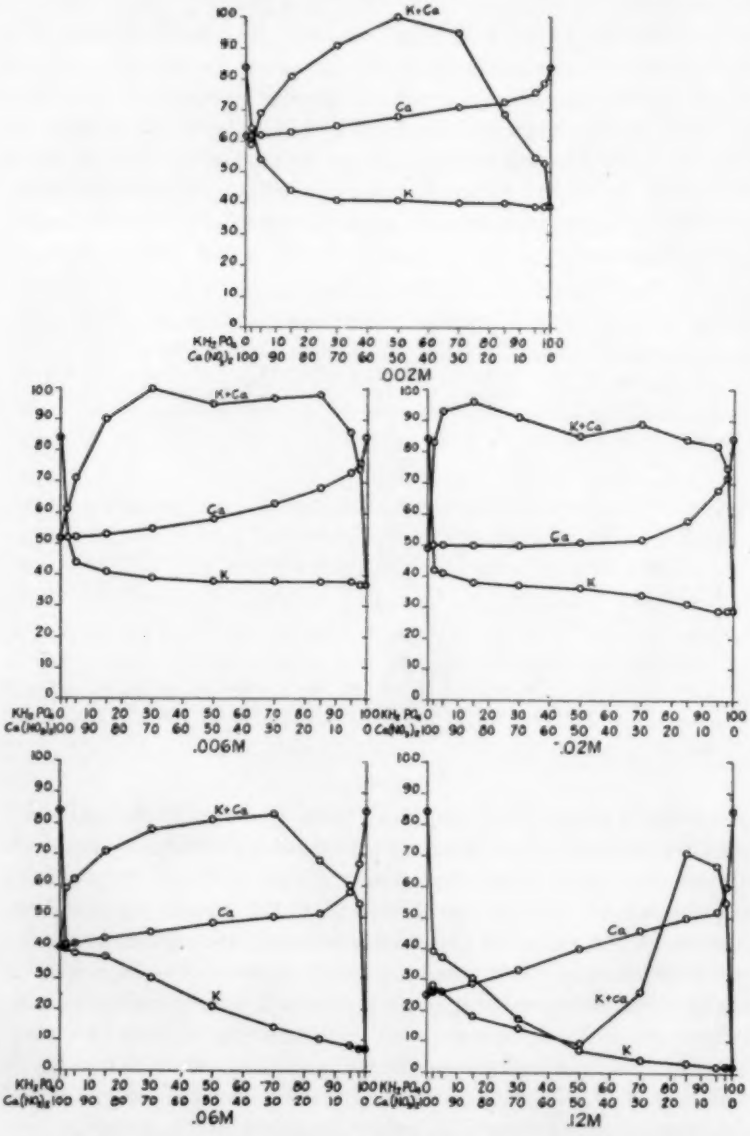

Fig. 2. Elongation of wheat roots in mixed solutions of KH_2PO_4 and $Ca(NO_3)_2$ of five total molecular concentrations, and in simple solutions of these salts in the concentrations in which they exist in the mixed solutions. Ordinates represent percentages of elongation for standard solution; abscissas represent molecular proportions.

An examination of figure 2 shows that the critical ranges are very narrow and well defined for the three intermediate total concentrations (0.006 M, 0.02 M, and 0.06 M). In these cases the critical ranges include sets of salt proportions in which the partial concentration of one of the salts usually does not exceed 2 or 5 per cent of the total concentration; when this very low concentration is exceeded, the range of indifference is reached, where relatively large differences in salt proportions are not accompanied by corresponding differences in growth. For mixtures of the lowest total concentration (0.002 M), the critical ranges are not clearly defined, since the growth rate rises gradually to a maximum with a solution containing 50 per cent of each of the two salts. And for mixtures of the highest total concentration (0.12 M) the curve is quite different in shape from the others.

Optimum and nearly optimum salt proportions. A conspicuous feature of figure 2 is the general absence of clearly defined optimum salt proportions, since the growth rates are nearly the same throughout a wide range of salt proportions. From theoretical considerations, it is probable that under a given set of experimental conditions an optimum set of proportions could be determined if a sufficiently large number of plants were used in each culture and if the gradations in salt proportions were made sufficiently small. The relations brought out in figure 2 indicate, however, that the growth rates in the hypothetical optimum solution would be only slightly greater than that in in any one of many solutions differing considerably in the relative proportions of the two salts.

The observed maximum growth value with mixtures of the lowest total concentration is shown for a mixture in which 50 per cent of the dissolved molecules are KH_2PO_4 (and in which 50 per cent are of course $Ca(NO_3)_2$). The corresponding maximum for the next higher total concentration is indicated for either a mixture containing 30 per cent KH_2PO_4 or one containing 85 per cent KH_2PO_4; and with the successively higher concentrations the maxima are for 15, 70, and 85 per cent KH_2PO_4, respectively. With the exception of the mixtures of the highest total concentration (where a clear maximum is indicated for 85 per cent KH_2PO_4), there is no evidence that maximum growth would be associated with either an excess of KH_2PO_4

or of $Ca(NO_3)_2$. The general indications of figure 2 are that maximum growth may be obtained with a solution having approximately equal proportions of the two salts.

An idea of the range of approximately optimum salt proportions may be obtained if any growth rate which equals 85 per cent or more of the maximum growth rate for a given series is defined as optimum. By defining optimum growth in this way, minor fluctuations, due to unknown or uncontrolled experimental conditions, are not given too great a significance. A study of the data from this point of view shows that 85 per cent or more of the maximum growth rate was secured with 54 per cent of the possible range of proportions of KH_2PO_4 to $Ca(NO_3)_2$ for the lowest total concentration, and with 83, 93 67, and 15 per cent for successively higher concentrations. The data thus secured serve to emphasize the general relations evident in figure 2. The broad range of nearly optimum salt proportions is clearly shown by these values, and there is an indication that, as the total concentration increases, the range of salt proportions allowing approximately optimum growth becomes wider up to 0.02 M and then becomes narrower.

Antagonism. Whether or not antagonism exists in mixed solutions of salts is indicated in most cases by the relative positions of the three curves shown in figure 2 for each set. These represent: (1) the growth rates in the mixed solutions of KH_2PO_4 and $Ca(NO_3)_2$, (2) the growth rates in simple solutions of KH_2PO_4 at concentrations equal to the concentrations of this salt in the mixed solutions, and (3) the growth rates of the corresponding simple solutions of $Ca(NO_3)_2$. By the simplest criterion, antagonism is considered to exist when the growth rate in the mixed solution is more rapid than in the more toxic single-salt solution—that is, when the curve for growth in the mixed solution is above the lowest curve for the simple solutions.

By this criterion it is evident that marked antagonism is indicated for nearly all of the mixed solutions illustrated in figure 2. The only exceptions are the solutions of the highest total concentration (0.12 M) in which KH_2PO_4 constitutes less than 50 per cent of the total concentration.

Antagonism is illustrated in a conspicuous manner by the fact that for all but the highest total concentration (0.12 M) the curve representing the growth rates in the two-salt solutions

extends for a considerable portion of its range above the higher of the two curves for the single-salt solutions. For these ranges, growth in the mixed solutions is not only more rapid than in the more toxic single-salt solution, but it is also more rapid than in the less toxic simple solution. Mutual antagonism is thus brought out in a striking manner.

Quantitative estimation of antagonism. Osterhout (1914a, 1914b, 1915, 1918) was the first to attempt to estimate antagonism in a quantitative manner. As he has pointed out, antagonism may exist even if the growth rate in the two-salt solution is less rapid than in the more toxic single-salt solution; and for an exact quantitative measurement of antagonism, it is necessary to determine the 'additive effect'—that is, the total growth-retarding effect that the mixed solution would have if neither salt influenced the toxicity of the other salt. Antagonism may then be regarded as existing if the *actual* growth-retarding effect of the mixed solution is less than the 'additive effect'; and quantitative expressions of antagonism may be based upon a comparison of these two effects.

Only an approximation of the hypothetical additive effect can be obtained, since the calculation is based on the assumption that the effect of one salt can be expressed in terms of the other salt. This assumption would be entirely justified if the toxic effects of both salts were the same—if both salts affected the same physiological processes in the same qualitative manner—and if the quantitative effects were so related that for equal growth-retarding effects the concentration of the first salt was always a constant multiple of the concentration of the second salt.

The method here used in obtaining an approximation of the additive effect is essentially the same as that described by Osterhout (1915). It may be illustrated by means of an example. Suppose we are dealing with a mixture that contains 0.00030 M KH_2PO_4 plus 0.00170 M $Ca(NO_3)_2$. The additive effect may be obtained (1) by assuming that in the absence of antagonism the effect of the mixture would be the same as that of a sufficiently concentrated solution of KH_2PO_4 alone or (2) by assuming that in the absence of antagonism the effect of the mixture would be the same as that of a sufficiently concentrated solution of $Ca(NO_3)_2$ alone.

To express the additive effect in terms of KH_2PO_4 it is necessary to note from figure 1 that the growth in a solution of $Ca(NO_3)_2$ of the concentration (0.00170 M) in which this salt exists in the mixture is 63. What concentration of KH_2PO_4 allows this same growth? Examination of the curve for KH_2PO_4 shows that the concentration corresponding to a growth of 63 is 0.00004 M. Now, the concentration of KH_2PO_4 actually present in the mixture is 0.00030 M. If the $Ca(NO_3)_2$ acted like KH_2PO_4 the effect of the mixture would be that of 0.00004 M KH_2PO_4 + 0.00030 M KH_2PO_4, or 0.00034 M KH_2PO_4. The growth corresponding to 0.00034 M KH_2PO_4 is 43, and the growth-retarding effect is 100−43, or 57. The value 57 may be considered as a numerical index of the additive effect expressed in terms of KH_2PO_4.

The additive effect may also be expressed in terms of $Ca(NO_3)_2$. From the curve for KH_2PO_4 we see that the growth in a solution of KH_2PO_4 of the concentration (0.00030 M) in which this exists in the mixture is 44. Proceeding as before, the curve for $Ca(NO_3)_2$ shows that the concentration of this salt corresponding to growth of 44 is 0.04467 M. The concentration of $Ca(NO_3)_2$ actually present in the mixture is 0.00170 M. If the KH_2PO_4 acted like $Ca(NO_3)_2$, the effect of the mixture would be equal to that of 0.04467 M $Ca(NO_3)_2$ + 0.00170 M $Ca(NO_3)_2$, or 0.04637 M $Ca(NO_3)_2$. Since the growth corresponding to 0.04637 M $Ca(NO_3)_2$ is 43, the growth-retarding effect is 100 − 43, or 57, and the value 57 may be taken as a numerical index representing the additive effect expressed in terms of $Ca(NO_3)_2$.

It will be seen that the two numerical expressions for additive effect are the same. Differences, however, occur in many cases, since the slopes of the growth curves for KH_2PO_4 and $Ca(NO_3)_2$ are not alike when a logarithmic scale is used for concentrations.

Considering the additive effect of this mixture as 57, we may express antagonism in various ways. The actual growth-retarding effect of the mixed solution was found to be 100 − 81, or 19. Antagonism may be expressed simply as the difference 57 − 19, or 38. Or this difference may be expressed as a percentage of the additive effect. Accordingly, the numerical index of antagonism for the solution in question may be given as

$[(57-19)/57] \times 100 = 67$ per cent. When this method is employed, antagonism is considered as 0 per cent if the growth-retarding effect of the mixture is the same as the additive effect, and as 100 per cent if the growth-retarding effect of the mixture is 0 (that is, if growth in the mixture equals that in the standard solution).

Numerical expressions of the two values for the index of antagonism for each solution used in the present study are shown in table 6. Although the two values for each solution are not always alike, they tend to show a rather close agreement.

TABLE 6

Indices of antagonism in solutions of potassium dihydrogen phosphate and calcium nitrate

MOLECULAR PROPORTIONS		0.002 M SERIES		0.006 M SERIES		0.02 M SERIES		0.06 M SERIES		0.12 M SERIES	
KH_2PO_4	$Ca(NO_3)_2$	(K)	(Ca)	(K)	(Ca)	(K)	(Ca)	(K)	(Ca)	(K)	(Ca)
2	98	9	7	28	22	71	73	33	45	5	10
5	95	38	35	50	49	88	89	39	49	5	8
15	85	67	67	85	86	*93*	*94*	56	63	2	−3
30	70	85	85	*100*	*100*	86	86	69	71	4	9
50	50	*100*	*100*	92	92	76	78	77	78	2	9
70	30	92	92	95	95	83	84	*80*	*81*	23	26
85	15	48	48	98	98	77	77	64	65	*70*	*71*
95	5	25	25	77	77	75	75	57	57	66	67
98	2	22	22	60	60	65	65	51	52	55	55

Maximum indices of antagonism of 100 per cent are indicated for the two lowest concentrations, and the corresponding value for the next series is only slightly lower. A significant difference is apparent for the next higher concentration, and a further decrease is indicated for the highest concentration. If plotted, the data of all but the highest concentration would give rather round-topped, symmetrical curves, with maximum values corresponding to partial concentrations of KH_2PO_4 of 50, 30, 15, 70, and 85 per cent as the total concentration varied from 0.002 to 0.12 M, respectively. In general, these data suggest that antagonism follows the growth in the mixed solutions.

Mixed solutions of calcium nitrate and magnesium sulphate

General features of graphs. In general shape the curves of figure 3, representing growth of wheat roots in mixed solutions of $Ca(NO_3)_2$ and $MgSO_4$, tend to be round-topped or flat-topped and symmetrical, and they resemble closely those for KH_2PO_4 and $Ca(NO_3)_2$ discussed in the preceding section of this paper. Only in the case of mixtures of the highest total concentration (0.12 M) is there an indication of a distinct maximum. As the total concentration changes from 0.002 M to 0.12 M, a tendency may be noted for the curves to change from flat-topped to

TABLE 7

Growth in mixed solutions of calcium nitrate and magnesium sulphate

PROPORTIONS		.002 M SERIES			0.006 M SERIES			0.02 M SERIES			0.06 M SERIES			0.12 M SERIES		
$Ca(NO_3)_2$	$MgSO_4$	A	B	Av.	A	B	Av.	A	B	Av.	A	B	Av.	A	B	Av.
0	100			23			18			11			8			6
2	98	47	46	47	20	19	20	13	12	13	10	10	10	13	12	13
5	95	90	83	87	32	32	32	29	27	28	16	15	16	13	13	13
15	85	96	95	96	83	79	81	52	49	51	55	54	55	39	36	38
30	70	97	95	96	79	77	78	70	65	68	73	71	72	49	45	47
50	50	92	90	91	84	70	77	77	69	73	78	75	77	66	62	64
70	30	92	88	90	82	79	81	71	66	69	81	80	81	52	51	52
85	15	94	89	92	80	67	74	68	65	67	79	73	76	42	41	42
95	5	84	80	82	52	50	51	63	50	57	58	54	56	30	29	30
98	2	82	78	80	51	48	50	60	48	54	50	49	50	30	29	30
100	0			62			49			52			41			21

round-topped and finally to a form having a fairly clear peak. The curve for the strongest solutions (unlike that for the preceding pair of salts) is as symmetrical as those for lower total concentrations. As in the preceding series, the maximum growth rate tends to be lower as the total concentration increases. Thus, the curve for the lowest total concentration rises to an elevation closely approaching 100 (the growth of the roots in the standard solution), and the curves for the progressively lower concentrations rise to about 80, 70, 80, and 60, respectively.

Attention should be called to the fact that precipitates were formed in three of the mixed solutions having the highest

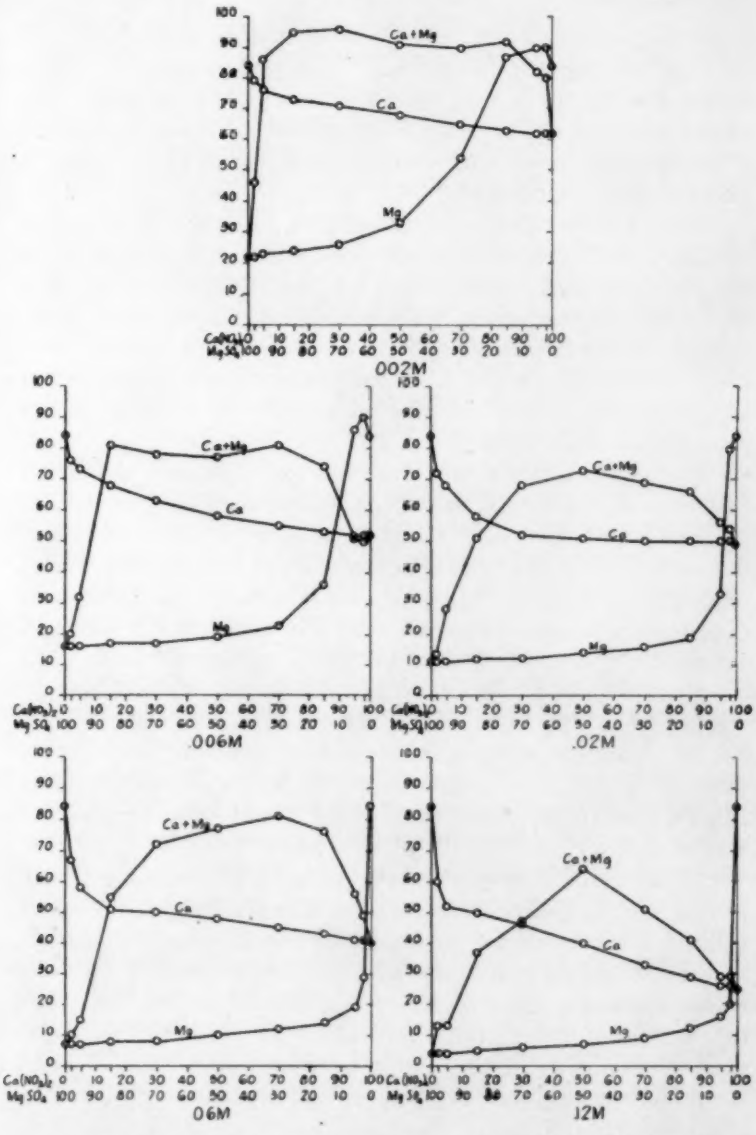

Fig. 3. Elongation of wheat roots in mixed solutions of $Ca(NO_3)_2$ and $MgSO_4$ of five total molecular concentrations, and in simple solutions of these salts in the concentrations in which they exist in the mixed solutions. Ordinates represent percentages of elongation for standard solution; abscissas represent molecular proportions.

total concentration (0.12 M)—namely, the mixtures having 30, 50, and 70 per cent $Ca(NO_3)_2$. The precipitation of $CaSO_4$ would reduce the actual concentration of the calcium ion in these solutions. The exact composition of these solutions is unknown, and hence the results obtained with them cannot be accurately interpreted.

Critical ranges and ranges of indifference. Each of the curves of figure 3, like those of figure 2, exhibits a steep slope at the right and left and a gradual slope in the middle portion. Thus, as in the former series, it is possible to distinguish critical ranges, within which growth is strongly influenced by small differences in salt proportions, and ranges of indifference, within which growth is nearly the same for wide variations in relative proportions of the salts.

The critical ranges are very narrow and well defined for mixtures of the lowest total concentration, but they tend to become wider and less distinct as the total concentration increases. And at a total concentration of 0.12 M they are not distinguishable; here the curve rises gradually to a peak for an equimolar mixture of the two salts and then falls gradually as the relative concentration of $Ca(NO_3)_2$ becomes greater.

Since the rise of the curves at the left side of the diagram is steeper than the rise at the right side, low proportions of $Ca(NO_3)_2$ appear to antagonize the toxic action of large proportions of $MgSO_4$ to a greater degree than low concentrations of $MgSO_4$ counteract the toxicity of high concentrations of $Ca(NO_3)_2$.

In the critical ranges the curves tend to rise more steeply as the total concentration of the mixture becomes lower (a relation not evident for the preceding pair of salts). Thus within the critical ranges at the left the effectiveness of $Ca(NO_3)_2$ in counteracting the toxicity of $MgSO_4$ is relatively greater in dilute than in concentrated solutions. Or, in order to counteract the toxicity of $MgSO_4$ and to produce *nearly optimal growth*, the molecular ratio of $Ca(NO_3)_2$ to $MgSO_4$ must be greater when the total concentration (or the concentration of $MgSO_4$) is greater. A similar relation is evident for the critical ranges at the right. However, when *optimal growth* is considered, there is no strong evidence that best proportions are different for the various total concentrations.

Optimum and nearly optimum salt proportions. Figure 3 agrees with figure 2 in showing broad ranges of nearly optimum salt proportions. Only in the case of the series of solutions having the highest total concentration (0.12 M) is there an indication of a pronounced optimum. We should expect that an optimum set of salt proportions could be determined if a sufficiently detailed study were made; but the shape of the curves indicates that the growth rate in the optimum solution would be only slightly greater than that actually observed with many solutions having considerably different proportions of the two constituent salts.

A maximum growth rate for mixtures of the lowest total concentration (0.002 M) is to be noted for a solution having 30 per cent $Ca(NO_3)_2$ (and therefore 70 per cent $MgSO_4$). The corresponding maxima for mixed solutions having higher total concentrations are shown for solutions having 10, 50, 70, and 50 per cent $Ca(NO_3)_2$, respectively. Only with the most concentrated mixture is a clearly defined maximum indicated. On the whole, a mixture having 50 per cent of each of the two salts appears to be nearly optimum.

By defining an optimum range of growth as in the preceding series (this range including growth rates which equal 85 per cent or more of the observed maximum), it is found that 90 per cent of the possible range of proportions of $Ca(NO_3)_2$ to $MgSO_4$ allows growth of this character in solutions of the lowest total concentration, and that for successively higher total concentrations the corresponding ranges of salt proportions are 75, 64, 60, and 26 respectively. As the total concentration increases, there is a decrease in the range of salt proportions allowing approximately optimum growth. The decrease is gradual until the concentration is changed from 0.06 to 0.12 M, when an abrupt decrease is evident.

Antagonism. Antagonism is clearly shown for all of the mixed solutions of $Ca(NO_3)_2$ and $MgSO_4$ illustrated in figure 3, since the curves for growth in the mixed solutions always lie above the lowest graph for the simple solutions. Mutual antagonism is brought out very distinctly, since the greater part of each curve representing growth in the two-salt solutions lies above the higher of the two curves of growth in the single-salt solutions.

Quantitative estimation of antagonism. Numerical indices of antagonism in mixed solutions of $Ca(NO_3)_2$ and $MgSO_4$ have been derived as in the preceding series, and these are shown in table 8. In general, they are somewhat lower than the corresponding indices for the preceding pair of salts. Agreement with the preceding series is seen in the fact that highest maximum indices of antagonism are shown for the mixed solutions having the lowest total concentration, and that much lower maximum values are shown for the most concentrated two-salt solutions.

TABLE 8

Indices of antagonism in solutions of calcium nitrate and magnesium sulphate

MOLECULAR PROPORTIONS		0.002 M SERIES		0.006 M SERIES		0.02 M SERIES		0.06 M SERIES		0.12 M SERIES	
$Ca(NO_3)_2$	$MgSO_4$	(Ca)	(Mg)	(Ca)	(Mg)	(Ca)	(Mg)	(Ca)	(Mg)	(Ca)	(Mg)
2	98	32	33	6	6	2	2	3	3	10	9
5	95	83	84	20	20	19	19	10	10	10	9
15	85	95	*95*	*77*	*78*	44	44	52	52	36	35
30	70	*95*	*95*	74	74	64	63	70	70	47	44
50	50	87	88	72	72	*69*	*69*	76	74	*64*	*61*
70	30	79	86	75	76	64	52	*80*	*78*	52	47
85	15	78	87	61	65	60	60	74	72	42	35
95	5	53	64	−2	25	40	43	50	47	30	18
98	2	47	55	−4	12	10	32	36	36	21	16

However, maximum values for intermediate total concentrations fluctuate, and do not form the regularly decreasing series noted for the preceding pair of salts.

Round-topped and nearly symmetrical curves would be obtained if the indices of antagonism were plotted as graphs. Maximum values would correspond to partial concentrations of $Ca(NO_3)_2$ of 30, 15, 50, 70, and 50 per cent as the total concentration increased from 0.002 to 0.12 M, respectively. The fluctuation here noted suggests that approximately maximum values may be obtained with a wide range of salt proportions.

Mixed solutions of potassium dihydrogen phosphate and magnesium sulphate

General features of graphs. The curves of figure 4, representing growth in mixed solutions of KH_2PO_4 and $MgSO_4$, agree closely in general form with those for mixed solutions of

KH_2PO_4 and $Ca(NO_3)_2$ and of $Ca(NO_3)_2$ and $MgSO_4$. They represent the same general type of round-topped symmetrical curves, but they rise somewhat more gradually than those of the preceding series. As the total concentration increases from 0.002 to 0.12 M, the shape of the curves remains practically constant. In contrast with the relations suggested by figures 2 and 3, there is no indication of a more pronounced peak for mixtures of the highest total concentration. In agreement with both of the preceding series, the maximum growth rate tends to be lower as the total concentration increases. The curves

TABLE 9

Growth in mixed solutions of potassium dihydrogen phosphate and magnesium sulphate

PROPORTIONS		0.002 M SERIES			0.006 M SERIES			0.02 M SERIES			0.06 M SERIES			0.12 M SERIES		
KH_2PO_4	$MgSO_4$	A	B	Av.	A	B	Av.	A	B	Av.	A	B	Av.	A	B	Av.
0	100			23			18			11			8			6
2	98	36	33	35	23	21	22	15	14	15	13	12	13	7	6	7
5	95	37	31	34	32	29	31	22	17	20	17	17	17	9	9	9
15	85	54	45	50	47	42	45	53	50	52	28	28	28	11	9	10
30	70	71	65	68	81	68	75	74	64	69	43	40	42	11	10	11
50	50	77	71	74	91	82	87	77	77	77	40	39	40	12	10	11
70	30	70	70	70	80	80	80	76	69	73	44	38	41	11	10	11
85	15	65	58	62	70	67	69	73	70	72	25	23	24	10	9	10
95	5	58	49	54	58	57	58	72	62	67	17	16	17	6	6	6
98	2	51	49	50	53	52	53	58	58	58	9	9	9	4	4	4
100	0			42			42			35			8			3

for the three lowest concentrations rise to approximately the same height, but the curve for 0.06 M rises to a much lower elevation and that for 0.12 M exhibits an extremely slight rise.

Critical ranges and ranges of indifference. Since most of the curves of figure 4 rise more gradually than those for the preceding series, the critical ranges of salt proportions are broader and the corresponding ranges of indifference are narrower. An exception to this statement is seen in the case of the most concentrated mixtures (0.12 M); here the critical ranges are very narrow and the range of indifference includes most of the salt proportions tested. This is in marked contrast with both of the preceding series; for in them the critical ranges are broadest for mixtures of highest total concentration.

The less steep rise of the curves at the left side of the diagram than at the right side indicates that low proportions of $MgSO_4$ are relatively more effective in antagonizing high proportions of KH_2PO_4 than are low proportions of KH_2PO_4 in counteracting the toxicity of high proportions of $MgSO_4$.

In agreement with the results obtained with the preceding pair of salts, the curves tend to rise more steeply in the critical ranges as the total concentration of the mixture becomes lower. In the critical range at the left, the molecular ratio of KH_2PO_4 to $MgSO_4$ must be greater to counteract the toxicity of the $MgSO_4$ when the total concentration (or the concentration of $MgSO_4$) is higher. And in the critical range at the right, the effectiveness of $MgSO_4$ in overcoming the toxicity of KH_2PO_4 is relatively greater in dilute than in concentrated solutions. Nevertheless, optimal proportions may be the same for all of the total concentrations tested.

Optimum and nearly optimum salt proportions. With the exception of the strongest solutions, the range of nearly optimum proportions appears to be narrower for mixtures of KH_2PO_4 and $MgSO_4$ than for those of the other pairs of salts. For all total concentrations maximum growth is indicated for mixtures having nearly equal molecular productions of KH_2PO_4 and $MgSO_4$.

Approximately optimum growth (85 per cent or more of the observed maximum) is indicated for 55 per cent of the possible range of salt proportions for the lowest total concentration, and for 49, 68, 53, and 80 per cent of this range for the successively higher concentrations. For the lower total concentrations these ranges are in general narrower than with the preceding pairs of salts; but for the highest concentration the range is much broader than with either of the preceding pairs. With mixtures of KH_2PO_4 and $MgSO_4$ no tendency is exhibited for the optimum range of salt proportions to decrease as the total concentration increases. Such a tendency was noted, however, for the other combinations of salts.

Antagonism. In agreement with the results for the other pairs of salts, striking evidence of antagonism is seen in figure 4, since curves for the two-salt solutions always lie considerably above the corresponding curves for the more toxic single-salt solutions. Moreover, mutual antagonism is clearly shown,

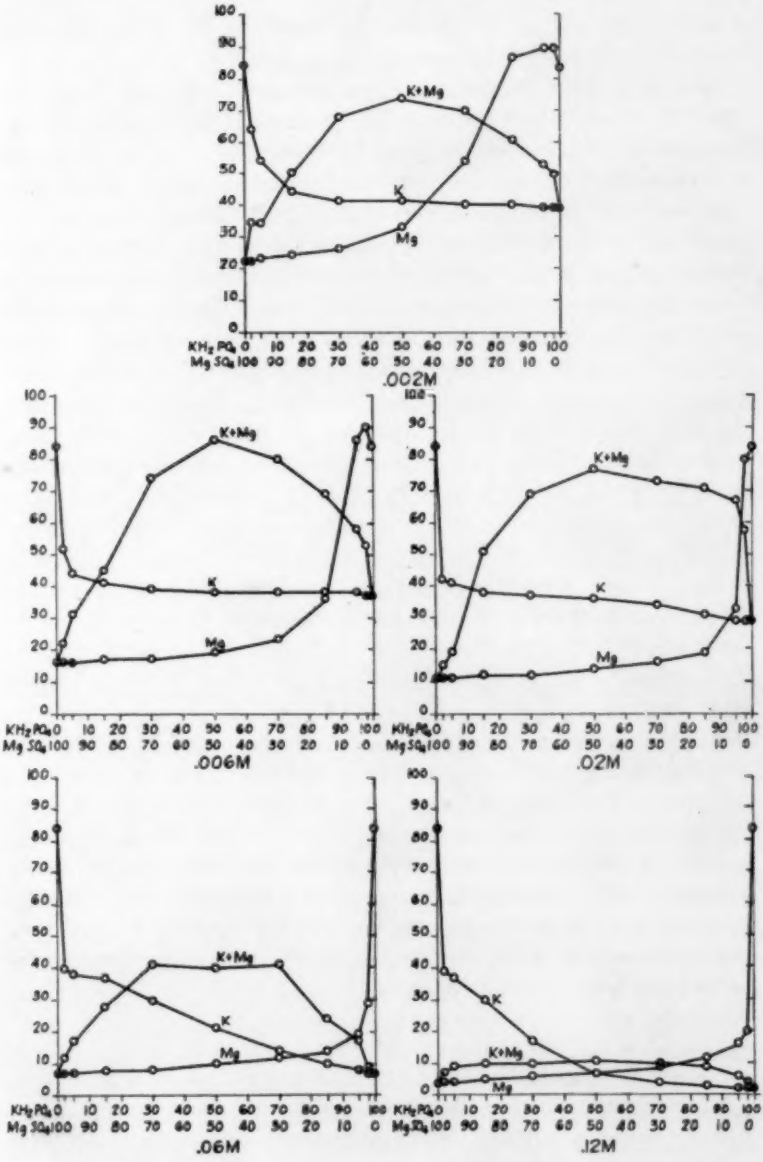

Fig. 4. Elongation of wheat roots in mixed solutions of KH_2PO_4 and $MgSO_4$ of five total molecular concentrations, and in simple solutions of these salts in the concentrations in which they exist in the mixed solutions. Ordinates represent percentages of elongation for standard solution; abscissas represent molecular proportions.

since a large part of each curve for mixtures lies above that for the less toxic single-salt solution.

Quantitative estimation of antagonism. In general, the numerical indices of antagonism between KH_2PO_4 and $MgSO_4$ (presented in table 10) tend to be somewhat smaller than the corresponding values for the other pairs of salts, and a conspicuous difference in this direction is evident with the two highest total concentrations, 0.06 and 0.12 M. The maximum indices of antagonism with these concentrations are markedly lower for mixed solutions of KH_2PO_4 and $MgSO_4$ than for the solutions of the other pair of salts.

TABLE 10

Indices of antagonism in solutions of potassium dihydrogen phosphate and magnesium sulphate

MOLECULAR PROPORTIONS		0.002 M SERIES		0.006 M SERIES		0.02 M SERIES		0.06 M SERIES		0.12 M SERIES	
KH_2PO_4	$MgSO_4$	(K)	(Mg)	(K)	(Mg)	(K)	(Mg)	(K)	(Mg)	(K)	(Mg)
2	98	17	19	8	8	4	4	6	6	3	3
5	95	14	18	19	19	10	10	12	11	5	5
15	85	34	38	35	35	46	45	24	23	7	6
30	70	57	60	70	70	65	65	*40*	*37*	8	6
50	50	*62*	*66*	*84*	*84*	*74*	*74*	38	34	*9*	7
70	30	50	59	75	75	70	68	39	34	*9*	*8*
85	15	37	45	54	69	69	66	22	16	8	7
95	5	23	28	32	40	60	58	14	10	4	3
98	2	17	18	24	29	41	45	5	2	2	1

As in the preceding series, the round-topped and fairly symmetrical curves would be obtained if these data were plotted as graphs. Maximum values would be shown for partial concentrations of KH_2PO_4 of 50, 50, 50, 30, and 50 per cent, with increasing total concentrations.

SUMMARY

A study was made of the rate of elongation of wheat roots supplied with simple solutions of potassium dihydrogen phosphate, calcium nitrate, and magnesium sulphate, and with solutions containing pairs of these salts. The single-salt solutions ranged in concentration from 0.00004 M to 0.24 M. For each pair of salts, five series of cultures were used. These series

represented total concentrations of 0.002 M, 0.006 M, 0.02 M, 0.06 M, and 0.12 M (corresponding roughly in osmotic value to 0.1, 0.3, 1, 3, and 6 atmospheres). With each total concentration eleven different sets of salt proportions were tested. Wheat seedlings having roots with an initial length of about 6 mm. were placed in the culture solutions and grown in darkness at a temperature of about 20°C. Each test was terminated when the roots in a standard solution had attained a length of 90 mm., the period thus required being about 100 hours. Amounts of root elongation, expressed as a percentage of the elongation in the standard solution, are used as the basis for quantitative comparisons of the physiological effects of the culture solutions. The principal results and conclusions follow:

1. Except in very dilute solutions of $MgSO_4$, the rate of root elongation was lower in single-salt solutions than in distilled water (prepared with a Barnstead still). Elongation was inversely related to concentration, but no simple rule could be formulated regarding the relationship of toxicity to concentration, over the wide range of concentration tested.

2. The order of toxicity of the three salts was different for different degrees of toxicity. For slight growth retardation, the order of toxicity of the three salts (from most to least toxic) is KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4$. For medium retardation, the order is KH_2PO_4, $MgSO_4$ $Ca(NO_3)_2$; and for high retardation, the order is $MgSO_4$, KH_2PO_4, $Ca(NO_3)_2$. Differences between the effects of the three salts are also shown by the fact that the growth curves have different slopes when concentrations are plotted on a logarithmic scale; thus equally toxic solutions of the three salts do not remain equally toxic when diluted to the same degree. Specific toxic effects are further indicated by differences in the appearance of roots grown in simple solutions of the three salts.

3. The typical curve representing growth in two-salt solutions, with a wide range of salt proportions and a constant molecular concentration, tends to be round-topped and symmetrical, with a maximum corresponding to approximately equal proportions of the two salts. The maximum, however, was not sharply defined in most cases. The typical curve tends to exhibit a steep slope at the extreme left and the extreme right and a very gradual slope in the middle. The steep por-

tions correspond to solutions in which the partial concentration of one of the salts is relatively low. Thus, for convenience of analysis, critical ranges of salt proportions may be distinguished, in which a slight difference in the ratio of one salt to the other is correlated with a very pronounced difference in the growth rate.

4. With a given set of salt proportions, the growth in mixed solutions was generally inversely related to total concentration, and this relation was similar for the three pairs of salts.

5. The wide range of salt progortions which allow nearly optimum growth is one of the conspicuous features of these results. It is prominent for solutions of each of the pairs of salts and for solutions varying in total concentration from 0.002 M to 0.12 M—that is, for solutions ranging from approximately one-tenth to six times the concentration commonly employed in nutrient solutions for higher plants. Optimum salt proportions probably could be defined precisely if a sufficiently detailed study were made, but the growth rate in the optimum solution would be only slightly higher than in many solutions differing considerably with respect to the relative proportions of their constituent salts.

6. Marked antagonism is indicated for nearly every one of the mixed solutions tested. The usual criterion of antagonism is the more rapid growth in the mixed solution than in a simple solution of the salt present in the more toxic concentration. For many of the solutions mutual antagonism was clearly shown by the fact that growth in the mixed solution was more rapid than in a simple solution of the less toxic salt.

7. To produce *nearly optimum growth*, the molecular ratio of $Ca(NO_3)_2$ to $MgSO_4$ had to be greater when the concentration of $MgSO_4$ was greater. Similar relations were evident with respect to the effect of $MgSO_4$ on the toxicity of $Ca(NO_3)_2$ and KH_2PO_4, and for the effect of KH_2PO_4 on the toxicity of $MgSO_4$. Nevertheless, to produce *optimum growth* the salt proportions apparently had to be the same for various total concentrations.

8. Quantitative indices of antagonism were calculated for all of the mixed solutions according to Osterhout's method of using the additive effect. In general, the index of antagonism was found to be inversely related to the total concentration of the solution. For solutions of a given total concentration,

maximum indices of antagonism were higher for mixtures of KH_2PO_4 and $Ca(NO_3)_2$ and of $Ca(NO_3)_2$ and $MgSO_4$ than for mixtures of KH_2PO_4 and $MgSO_4$.

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Long time experiments with plants in closed containers

RAYMOND H. WALLACE

(WITH A TEXT FIGURE AND PLATE 9)

It has long been known that the two fundamental processes of green plants, photosynthesis and respiration, are opposite in character and may be very nearly equal in the volumes of gases used and liberated. Some years ago, while performing experiments on the effects of illuminating gas upon green plants, I observed how easily the gas volume relation between these two processes can be demonstrated. In one of the experiments the plants remained sealed for more than a month, and were still as fresh and green as when first enclosed. Further experiments were then made to perfect the seal and properly control the moisture relation. Hermetically sealed containers were devised in which the plants received only heat and light from the external environment.

The original experiments in which the gas relations of green plants were discovered marked the beginning of modern experimental studies of plant metabolism, and as the full scope of these early experiments has been overlooked it seems worth while to summarize them in connection with an account of my own work and the special containers I have devised. I have examined the original records with reference to the question of just how long plants of various species and stages of development have been kept in sealed containers, and the details of the methods used for control of the moisture and other possible factors.

The possible length of time that a green plant can live in a sealed container is of interest as bearing on the possibility that factors other than the oxygen and carbon dioxide ratio may be concerned in the relation of a plant to its atmospheric and soil environment. Also the question whether the complete life cycle from seed to seed or spore to spore can be completed in such a limited environment needs further study.

In 1774 Priestley, the discoverer of dephlogisticated air (oxygen), reported that green plants possess a faculty of restoring air which had been fouled by the burning of a candle,

and attributed this purification to an imbibition by the plant of the phlogistic matter which had arisen from the inflammable matter. This preliminary discovery was followed soon by the work of Ingen-Housz (1779), which first put our knowledge of the gas relations of green plants on an experimental basis. He found that all green plants 'purify' the air in light, but 'foul' it in darkness. The 'purification' was found to take place only in the chlorophyll bearing portions of the plants, while the non-green parts such as roots, fruits, flowers, etc., gave off fixed air (carbon dioxide) at all times irrespective of the light conditions. Ingen-Housz (1796) was, however, apparently yet unaware that plants might be able to live in a closed system, since he says, 'A plant shut up in vacuo soon dies; and it dies in all sorts of aerial fluids which are incapable of supporting animal life—such as fixed air [carbon dioxide], inflammable air [methane], phlogisticated air, or azote [nitrogen], &c.'

De Saussure (1804), however, concluded from his very careful experiments that many green plants can live confined not only in common air, but in atmospheres consisting of pure nitrogen, pure hydrogen, or pure carbon monoxide. Even a vacuum made and maintained at 1.6 mm. had no harmful effects on many species. For all of these closed systems it was found necessary to exercise care not only in the choice of the species of plant, but also with respect to the intensity of the light to which the plants were exposed during the period of confinement. From his experiments he reached the basic conclusion that plants may be able to derive some oxygen from their own tissues by the conversion of the carbon dioxide from their respiration into oxygen by the action of light. He says, in connection with this diurnal and nocturnal gas relation, 'They made then total replacement during the day of that which they used during the night.'

De Saussure reports having kept *Lythrum Salicaria*, *Epilobium hirsutum*, and *Polygonum Persicaria* for months in light in atmospheres of common air, pure nitrogen, pure hydrogen, pure carbon monoxide, and even in a vacuum which was renewed daily. A concentration of carbon dioxide greater than 8.3 per cent was found to be fatal to most of the plants. He also found that a cactus, *Opuntia*, which had lost its vigor after having been in sunlight in a vacuum for more than a month,

recovered completely when transplanted to the garden, thus showing that the tissues of the plant were not permanently injured. The longest period that he reports having kept a plant in a closed system was four months. The plant in this case, *Lythrum Salicaria* had not lost a single leaf during this long period of confinement. He reports that a plant of another species, *Polygonum Persicaria*, which he kept in a vacuum that was renewed daily, showed at the end of six weeks no apparent injury, if one excepts two or three yellow leaves near the base of the plant.

On the other hand, he found that certain species seemed to be unable to maintain a healthy condition when kept in a closed chamber. Pea plants four or five days old could live for months in nitrogen, but were not healthy. Similar plants died when placed in a vacuum. Periwinkles (dwarf) could maintain themselves in common air or nitrogen for only about four weeks and then died, he believed, because of the excessive humidity. He found it necessary to protect the plants from even the weak direct rays of the sun when they were in a vacuum, but only strong direct rays were harmful to those growing in atmospheres of common air or nitrogen.

As the investigators who followed De Saussure were more interested in other phases of the problem of the gas relations of plants, we have heard nothing further concerning the growth of plants in closed containers until very recently. In this interim Boussingault, Schloesing, Maquenne, Demoussy, and others, worked upon the numerical values of the gas ratios in respiration and photosynthesis, and found them to be near unity, as would be expected from the results of De Saussure's experiments.

Livingston (1909) reported upon a fern growing as a volunteer in a sealed jar containing a soil sample in the Rothamstead Experiment Station at Harpenden near London. This soil sample, about one liter, had been collected in 1874 and preserved without drying in a large-mouthed, two-and-one-half-liter bottle. The fern, *Asplenium nigrum*, was first observed in 1903, but had doubtless been growing many years. A resealing at this time over the seal already present made it perfectly certain that there had been no interchange of materials between the interior and exterior of the bottle during the six years previous to the report (1909).

Transeau (1911) published a brief note on what he terms a microcosm, in which an alga, *Scenedesmus quadricauda*, and a goldfish were sealed in a six-and-one-quarter-liter jar containing about three liters of water and nutrient solution. In this system the fish was supported by the alga, and the latter in turn maintained itself and perhaps used the waste products of the fish. The experiment ran from January 1 until July 28, when an accidental exposure of the system to sunlight resulted in the death of the fish and the termination of the experiment.

Recently Maquenne and Demoussy (1921–23) have demonstrated that adult leaves of *Aucuba* can be detached from the plant and conserved in a vacuum for more than a year, if care is taken to expose them to light adequate for photosynthesis. They say nothing to indicate that they were aware that De Saussure had made similar experiments upon various entire plants more than one hundred years before. They report further that the leaves of bay cherry, privet, and *Euonymus* were all dead when removed from the vacuum after six months. An *Aucuba* leaf, on the other hand, sealed from May 6, 1921 to May 5, 1922 decomposed 2.4 cm. of carbon dioxide, and evolved an equal volume of oxygen when placed in the light for six hours. A cytological study (Dangeard and Dangeard 1923) of an *Aucuba* leaf, after it had been in a relative vacuum for a year, showed the leaf to be normal in all respects, as indicated by the reaction of the cells to intravitam stains, by protoplasmic streaming, and the general appearance of the cellular structures.

Maquenne and Demoussy believe among other things that the leaves must be full-grown if they are to survive in a vacuum, otherwise they continually lose gaseous or solid material through the formation of some fixed product such as cellulose. The loss of available materials to the plant must in time affect the ratio of carbon dioxide evolved to oxygen absorbed. This CO_2–O_2 ratio must be equal to or greater than one for the period of the experiment. They found that it was greater than one for leaves of *Aucuba*, since the vacuum tubes contained at the end of the experiment (in light) a quantity of oxygen about the same as one would find in tubes of the same size full of ordinary air. This is in agreement with the observations of De Saussure for entire plants. He found a vacuum or atmospheres made up as pure nitrogen, pure hydrogen, etc..

always showed a small amount of oxygen on analysis at the end of experiment. Maquenne and Demoussy also found that the period during which leaves could live in a vacuum was closely related to a ratio 1/N. In this ratio 1 is the volume of carbon dioxide liberated per hour by 100 gm. of leaves preserved in vacuo in darkness, while N represents the volume of carbon dioxide given off in darkness by an equal weight of similar leaves freshly picked. Leaves that maintain a ratio equal to or greater than unity and hold this value almost constant for a sixteen hour period can live for a long time in a vacuum, while those that do not soon die of starvation, because the carbon dioxide evolved in respiration is insufficient to maintain their photosynthesis. For sorrel, a plant that can survive for only a short time in vacuo, this ratio of 1/N was only 0.33 at the end of four hours, while at the end of sixteen hours it had dropped to zero. The ratio for *Aucuba* leaves, on the contrary, was 1.38 initially and was still 1.18 after sixteen hours. These specific differences may be associated with the rate of growth or the general hardiness of the tissues of the two species.

In the experiments which I have so far made, my attention has for the most part been directed to the practical phases of the problem of maintaining living plants in closed containers full of common air. To accomplish this one must consider three principal factors, namely, the form of container and method of sealing, the maintenance of soil and moisture conditions, and the kinds of plants best suited to life in closed containers. I have used 500 watt electric light bulbs very successfully as containers. These bulbs have a capacity of about 750 cc. The soil is placed in a short stemmed thistle tube whose base is embedded in a plaster of Paris foot. The stem of the thistle tube is filled with soil, which is in contact with the plaster of Paris foot as shown in text figure 1. About 10 cc. of water are placed in the apparatus. The water which is evaporated from the soil and plant condenses on the inside of the bulb, and runs down to the base, where it collects on top of the plaster of Paris. The capillarity of the soil and plaster of Paris insures a continuous and adequate supply of water to the plant. The same water is used over and over repeatedly by the plant. The base of the apparatus is usually a heavy pressed cup into which the

other parts are sealed. I have found that a layer of plaster of Paris over which is poured a layer of molten sealing wax or gutta percha is quite satisfactory as a seal.

Several kinds of soil have been tested to find those most suitable for these cultures of sealed plants. In my earlier experiments ordinary garden soil which had been prepared for

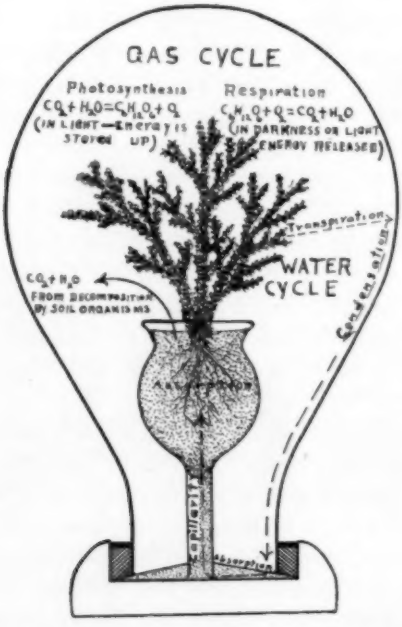

Fig. 1. Optical section of apparatus used for controlling the gas and moisture relations of plants in hermetically sealed cultures (For full explanation see text).

greenhouse use by the addition of small amounts of artificial fertilizer (dried blood and bone meal) was used. This use of fertilizer was later discontinued, however, since it was apparently not only unnecessary but often actually harmful, in that it stimulated the production of bacteria which were directly or indirectly injurious to the plants. Sterile or practically sterile soils have likewise proved unsatisfactory for use in these cultures. Garden soil sterilized by heat or merely allowed to dry thoroughly in the laboratory was far inferior to the same

soil kept in moist condition. A soil consisting of equal parts of quartz sand and clay-loam was almost as satisfactory as the unsterilized garden soil. The soil should contain some humus and the required nutrient salts as well as the organisms generally present in soils. Although the subject is as yet obscure, it seems probable that the soil organisms may have distinctly beneficial effects on plants, perhaps in returning carbon dioxide to the air by decomposition of the cellulose in the soil, or in furnishing some essential nutrient substances to the plants. Attempts to increase the possible growth of the plants by using soils rich in humus were unsuccessful.

I have experimented chiefly with twelve species of plants.[1] Four of them I have been unable to keep alive, namely: *Oxalis stricta*, *Begonia semperflorens*, *Impatiens sultani*, and *Vinca rosea alba*. All these species became diseased and died within a few weeks after sealing. They may be species that cannot survive in the high humidity always present in these systems, as was suggested by De Saussure for *Vinca*. It is not impossible that, as claimed by Maquenne and Demoussy for sorrel, they are unable to live because they do not evolve enough carbon dioxide in their respiration to maintain their photosynthesis. It is possible of course that the soil conditions or soil organisms may have been a factor in causing their death. All these possibilities need further study.

I have been able to keep four species, *Coleus Blumei*, *Bryophyllum calycinum*, *Nicotiana Tabacum*, and *Mimosa pudica*, alive for months when sealed hermetically, but with progressive deterioration. There was a great loss of leaves by abscission or by death without abscission. New leaves replaced those lost, but this in time seems to exhaust the plants or perhaps leads to the exhaustion of the surrounding atmosphere. Very frequently a plant may put out new leaves every few weeks to replace those which have died, but these new leaves are progressively smaller until the plant finally dies. Many plants, such as *Mimosa*, when taken from greenhouse conditions and sealed, quickly lose all their leaves, and then grow new ones apparently better adjusted to the conditions of the new environment.

[1] A popular account of these experiments has been given in the Scientific American **135**: 190–191. 1926.

A plant of *Mimosa pudica* with seven leaves, which was enclosed on March 24, illustrates both of these types of replacement of leaves. The first leaves of this plant began to discolor within two days after sealing and all had fallen four days later (March 30). Seven days later (April 6, fig. 2) the plant had put out a new set of six vigorous leaves. The old dead ones had undergone abscission and had fallen to the bottom of the apparatus. The new leaves remained fresh and green for three weeks, and exhibited the usual diurnal movements as well as movements in response to jarring. This set was then replaced by four new leaves, and these in turn by a third set. This plant finally died after having been sealed for four and one half months.

A plant of *Nicotiana Tabacum* shown in figure 3 lived for a similar length of time. This plant had only three leaves when enclosed on March 26. On April 6 the two older leaves were yellow, while the remaining one had grown vigorously, and a new one had made its appearance. It was photographed two weeks later (April 20, fig. 3) when it had grown an additional leaf. As will be seen in the figure, one leaf was entirely dead and drooping. One of the remaining leaves was very yellow, though it appears normal in the picture. This plant continued to grow new leaves and to lose old ones until August 10, when it was killed by an accidental exposure to direct sunlight. At the time of its death it had fifteen leaves in all, only two of which were green, while the others were in all stages of disintegration.

Four species, *Thuja occidentalis*, *Woodwardia orientalis*, *Dryopteris normalis*, and *Selaginella Emiliana*, have proved very well adapted to life in closed containers. Many cultures of these plants have remained for months in perfect condition. A plant of *Dryopteris* lived for twenty-two months, and then died because of loss of water through a broken seal. This plant, as it appeared after six weeks in the container, is shown in figure 1, plate 9. Originally the plant had only fifteen leaves, but after twenty months there were forty, only five of which were still green, the rest being in various stages of decay. Perhaps the most striking case of successful development in a closed container which I have observed is that of a plant of *Woodwardia orientalis*. This plant, with nine leaves, was enclosed on August 10, 1926, and at present, July 27, 1928, it

has lost only three leaves and has grown five new ones. The plant appears as vigorous and healthy now as on the day it was put in the container. A plant of *Selaginella* put in a container at the same time has lost two small branches, but the rest of the plant is in perfect condition.

These preliminary cultures show that the more or less balanced gas relation in photosynthesis and respiration is by no means the only factor determining the survival of plants in closed containers. The tolerance of a plant for such conditions seems to bear no relation to its position in the plant kingdom, since tolerant species have been found in widely separated groups. I have found that shade-loving plants are better adapted to live in closed containers than sun-loving plants, because of the heat relation. *Nicotiana* or *Mimosa* can live for only a few days unless exposed to rather strong diffused light; while a fern or *Selaginella* may die or at least become bleached under the same light, but will remain fresh and vigorous in weak diffused light. The sun plants as a group are unsatisfactory because the heat that accompanies the greater light intensity is imprisoned by the bulb and tends to injure the plants. Strong direct sunlight is fatal to all species which I have tested.

So far no one has been able to bring a green plant to the production of viable seeds or spores and thus complete its normal life cycle. There is, however, no theoretic impossibility of demonstrating such a complete life cycle in properly adjusted containers of sufficient size.

Further experiments are planned to determine whether cyclic changes in the gas relation occur and whether minute amounts of deleterious products are given off by the plants. Additional experiments are needed on the relation between rapidity of vegetative growth and survival value in closed containers, and comparative studies on the degenerative changes in plants of various types and ages when sealed in closed containers with relatively small volumes of gas.

It is a pleasure to express my appreciation to Professor R. A. Harper for his valuable criticism and helpful suggestions during the progress of these studies and the preparation of this paper.

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[2] The rarity of this Essay is indicated by Julius Wiesner in his biography of Jan Ingen-Housz (Wien, 1905), where he states that since this important original publication was not to be found in the libraries of the Continent, he secured the citation of the copy in the British Museum (Bloomsbury), where it appears as no. III in a collection bearing the title: Additional Appendix to the Outlines of the Fifteenth Chapter of the Proposed General Report from the Board of Agriculture. The copy of the Essay (without title page) which has been consulted in connection with this paper is found in the library of the U. S. Department of Agriculture, in a volume in which the *Outlines of the Fifteenth Chapter* is preceded by: Heron, Robert. General view of the natural circumstances of—Hebrides. Edinburgh, J. Patterson, 1794. This volume bears on the outside the library title: Agriculture of the Counties of Britain, vol. 14.

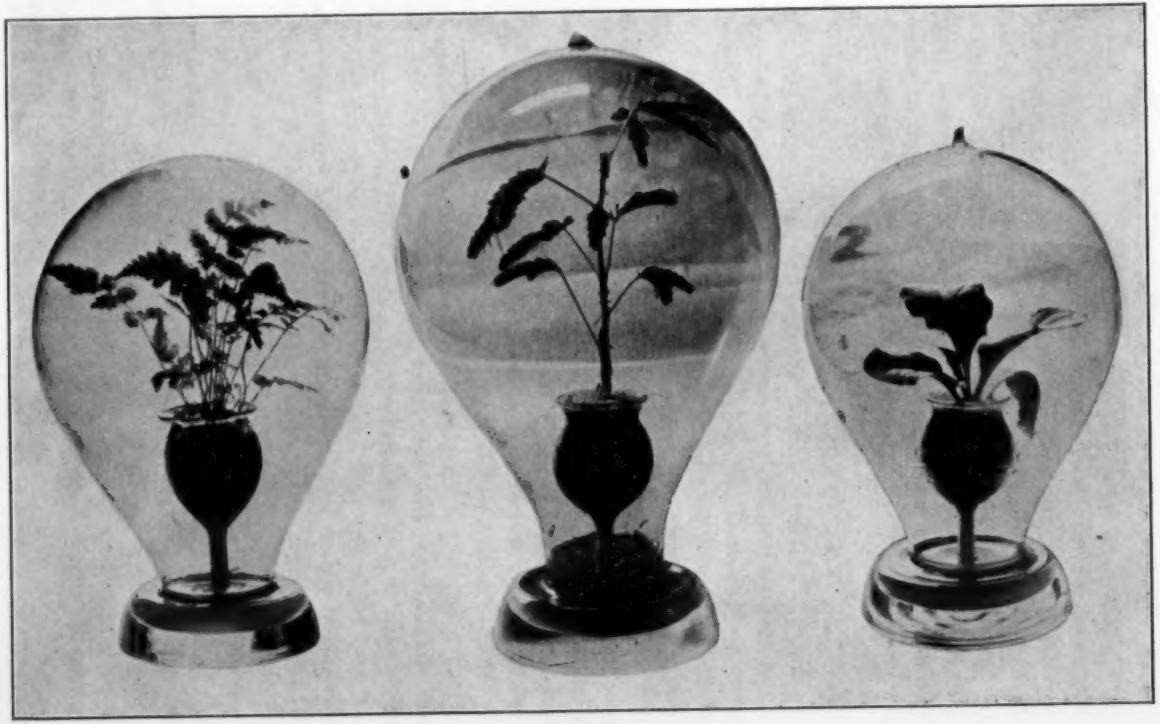
Explanation of plate 9

Each container has a capacity of about 750 cc. (500 watt bulb). The three figures vary slightly from 1/3 natural size.

Fig. 1. Culture of *Dryopteris normalis*, six weeks old. One leaf has died and three new ones have appeared. This plant was still alive after twenty-two months.

Fig. 2. Culture of *Mimosa pudica*, about two weeks old. The existing set of six vigorous leaves has grown since the plant was enclosed. The original set of seven leaves fell a few days after sealing and can still be seen in the base of the apparatus. This plant died after being sealed four and one-half months.

Fig. 3. Culture of *Nicotiana Tabacum*, twenty-five days old. One leaf is dead, one is discolored and two new ones have made their appearance. The plant died after four and one-half months.



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WALLACE: PLANTS IN CLOSED CONTAINERS

U.S. DEPT.

Notes on Scrophulariaceae of the northwestern United States

FRANCIS W. PENNELL

In collections of Scrophulariaceae from the northwestern United States recently submitted for identification are several species that have proved to be new to science. Although at some future date the writer hopes to visit this area and to attempt a summarized study of all of this family therefrom, deference to the interest of those offering these specimens makes it desirable to publish certain notes now.

I. A NEW SCROPHULARIA FROM OREGON

During the spring and early summer of 1927 Professor Edward Maris Harvey of the Oregon Agricultural College made a considerable collection of the Scrophulariaceae growing around Corvallis or gathered on an excursion from there over to the Pacific coast at Newport, Oregon. His entire series was most generously given me, and will yield information on several problems dealing with species peculiar to the Oregon and Washington coast. Among these is a plant which has long passed as *Scrophularia californica* Cham. & Schlechtd., but which on climatic grounds alone would have a strong presumptive probability of distinctness. The latter species is widespread through most of California from the coast to the lower slopes of the Sierra, and from the arid southland to the hills approaching the Oregon border; evidently it is capable of enduring dry or very dry atmospheres. But the plant that has borne this name northward occurs only close to the coast from Oregon to British Columbia; it would seem indeed strange if a species occurring so widely through the dry climate of California should extend northward only in a narrow strip that holds strictly to the humid coastal belt. Comparison of the two plants shows morphological differences confirming our suspicion of distinctness; the northern plant does not possess the rounded sepals, the ample lax panicles, and the strongly doubly serrate-dentate leaf-blades of *S. californica*. It may be described as follows:

Scrophularia oregana Pennell, sp. nov. Stem 9–12 dm. tall, finely glandular-pubescent, more coarsely so above. Leaf-blades 9–11 cm. long, simply or somewhat doubly serrate-dentate, ovate or triangular-ovate, truncate or slightly cordate at base, finely pubescent beneath, on wingless pubescent petioles 2–5 cm. long. Panicle narrow, of three to five fascicles, the pedicels much shorter than the stout ascending primary peduncles. Sepals 3–4 mm. long, ovate or triangular-ovate, acute or acuminate, not scarious. Corolla 9–12 mm. long, red-brown, especially on the posterior lobes, the anterior lobes paler, greenish-yellow. Sterile filament clavate, scarcely wider distally, purple throughout or distally greenish. Capsule 7–9 mm. long, ovoid-conic, acuminate, brown. Seeds 0.8 mm. long.

Type, from 'dry open area, surrounded by dense Sitka spruce forest,' Newport, Lincoln Co., Oregon, collected in flower May 4–5, 1927, by Edward Maris Harvey *57*; in Herb. Academy of Natural Sciences of Philadelphia.

The following specimens are also in the Academy's Herbarium:

BRITISH COLUMBIA. Moist sandy gully, Renfrew, Vancouver Island, *C. O. Rosendahl & C. J. Brand 55.*

WASHINGTON. Chehalis Co.: Granville, *H. S. Conard 339*; and Hoquiam, *F. H. Lamb 1224.*

OREGON. Benton Co.: Blodgett, *E. M. Harvey 32.* Clatsop Co.: Shore of Columbia R., above Astoria, *Thomas Meehan.* Lincoln Co.: Newport, *E. M. Harvey 57.*

Beside this species, *Scrophularia lanceolata* Pursh[1] also occurs in Oregon and Washington. It is likewise frequently confused with *S. californica*, which it greatly resembles in habit. From both the latter and *S. oregana*, however, it may be easily distinguished by the sterile filament, which is greenish-yellow and wider than long, distally widely flaring. This organ in *S. californica* is as narrow as in *S. oregana* and seems to be constantly purple. The panicle of *S. lanceolata* is much elongated while it is narrow and with stout peduncles as in *S. oregana* rather than *S. californica*. The leaf-blades vary from slightly serrate to usually coarsely dentate, but are hardly doubly so as in *S. californica*. All inland records of *S. californica* from the northwestern United States pertain to *S. lanceolata*,

[1] The use of this name to replace *Scrophularia leporella* Bickn. has been explained in Torreya **22**: 81-82. 1922.

while it reaches the Pacific coast near the mouth of the Columbia River. *Scrophularia lanceolata*, from which *S. occidentalis* (Rydb.) Bickn. seems not distinct even varietally, has a singularly wide distribution, ranging far north and south through the Rocky Mountains and crossing the northern United States from the Pacific to the Atlantic Oceans. The following specimens from Oregon and Washington are in the Academy's Herbarium:

WASHINGTON. Chehalis Co.: Montesano, *A. A. & E. G. Heller 4003.*

OREGON. Marion Co.: Salem, *J. C. Nelson 3897.*

II. A NEW PEDICULARIS FROM WASHINGTON

In August, 1927, Mr. F. A. Warren, while botanizing over some of the less known portions of Mount Rainier National Park, found a *Pedicularis* strikingly different from any species known to him. After comparison with specimens at the Herbarium of the State College of Washington, Pullman, Wash., had failed to identify the plant, specimens were sent to me by Professor Harold St. John and the discoverer. A wider comparison has sustained their belief in the plant's distinctness; there seems to be no species closely akin to this among those of this genus in North America nor is there reason for suspecting that the plant can be matched among the hundreds that have been described from Asia and Europe, only the Arctic species of which have been carefully inspected. Rather does it seem that this plant, like *P. ornithorhynchos* Benth., will be found to be restricted to the upper slopes of Mount Rainier. These two species of *Pedicularis*, endemic to this one mountain, give us a hint of the rich flora of similarly localized species that must once have existed from the Washington to the Alaskan boundary and have been destroyed by the extensive Cordilleran glaciation of Pleistocene time.

From other species of the genus occurring in Washington, this differs in the combination of yellow corolla that has a beakless galea and tripinnatifid leaf-blades that are ovate in general outline. It may be described:

Pedicularis rainierensis Pennell & Warren, sp. nov. Stem 1–4 dm. tall, glabrous below the inflorescence. Leaves alternate, both radical and cauline, the former petioled, the latter shortly

so or sessile, the blades ovate in general outline, glabrous, pale and finely reticulate beneath, those of the radical and lower leaves 5–7 cm. long, 2–3 cm. wide, tripinnatifid, deeply cut (incisions reaching almost or quite to the midrib that continues as a narrow axis between the segments) into seven or nine pairs of segments which are ovate in outline, acute, in turn lobed and the lobes serrate, the terminal segment largest and most attenuate. Inflorescence nearly spicate, 3–15 cm. long, the rachis, bracts and calyces hirsute. Bracts mostly about 10 mm. long, lanceolate, entire, only the lowest elongate and with crenately lobed, slightly foliose blades. Pedicels less than 1 mm. long. Calyx 7–8 mm. long, the tube cleft more deeply medianly than laterally; the posterior sepal the shortest, as a free lobe triangular-attenuate, projecting 3 mm. from the posterior cleft of the calyx-tube; the lateral sepals even, those of each side united to within 3 mm. of apex, the free lobes lanceolate, acute. Corolla 15 mm. long, yellow; the posterior lip (galea) erect, distally decurved, at apex slightly narrowed and truncate (without lateral teeth); the anterior lip ascending or appressed, but so much shorter than the posterior as not to cover the orifice, the lobes erose. Filaments and anthers glabrous. Capsule 8 mm. long, with mucronate straight tip, the cells nearly equal, dehiscing only on the posterior side, the anterior cell opening by a longitudinal split of the septum. Seeds 2 mm. long, lunate-rhombic, dark brown, reticulate, the reticulations forming continuous longitudinal (or slightly spiral) lines, the cross-reticulations ladder-like.

Type, grassy meadows, near Indian Henry's, Mount Rainier National Park, Pierce Co,, Washington, collected in flower and fruit August 21, 1927, by F. A. Warren *537*; in Herb. Academy of Natural Sciences of Philadelphia; isotype in Herb. State College of Washington. Also seen from Van Trump Park, Mount Rainier National Park, collected in flower August 28, 1927, by F. A. Warren *538*; in the same herbaria.

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1928

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